Insulin-like Action of Chromate on Glucose Transport in Isolated Rat Adipocytes

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ABSTRACT—The effects of chromium compounds on 3-O-methylglucose (3-O-MG) transport were studied in isolated rat adipocytes. Sodium chromate significantly stimulated 3-O-MG uptake into adipocytes in a dose-dependent manner without altering the equilibrium space of 3-O-MG in adipocytes. The stimulatory effect reached the maximum at 300 μM, and the effect was 60–70% of the maximal insulin effect that was obtained with 20 nM insulin. The chromate concentration achieving a half-maximal effect was estimated at 50 μM. The effect of the combination of 1 mM chromate and 20 nM insulin was equipotent to that of 20 nM insulin alone, which showed that these two effects were not additive. The stimulatory effects of 1 mM chromate and 20 nM insulin were entirely abolished in adipocytes deprived of ATP, which indicated that these effects were completely ATP-dependent. Judging from experiments using various chromium compounds, CrO_4^{2-} was responsible for the insulinomimetic action. These results indicate that the action of CrO_4^{2-} is exerted through a mechanism analogous to that of insulin action, and that CrO_4^{2-} is a novel and useful tool for studying issues involved in insulin actions.

Keywords: Chromate, Glucose transport, Insulin action, Insulinomimetic agent

Vanadate, zinc compounds and selenate have been reported to exert insulin-like actions in vitro (1-5) and in vivo (6-9). We (10, 11) and other investigators (12) found independently new insulin mimickers, molybdate, and tungstate. We further observed that their peroxide form showed a more powerful insulinomimetic action (10). In the course of these investigations, we accidentally found that sodium chromate exerted an insulin-like effect on glucose transport with greater intensity than that of molybdate and tungstate (Y. Goto et al., unpublished data). Sodium chromate contains a hexavalent chromium (Cr^{6+}). We have no knowledge about the influence of Cr^{6+} on glucose utilization, but trivalent chromium (Cr^{3+}) is known to be a component of glucose tolerance factor that is involved in glucose tolerance in vivo (13). It would therefore be of value to study the action of chromate on glucose transport. The aim of the present study is to characterize the action of chromate on glucose transport using isolated rat adipocytes.

MATERIALS AND METHODS

Materials
Sodium chromate tetrahydrate and potassium cyanide were purchased from Wako Pure Chemical Industries (Osaka), chromium (VI) oxide, chromium (III) chloride hexahydrate and chromium (III) oxide were from Nacalai Tesque (Kyoto). The sources of the other materials were listed in our previous publications (10, 14).

Glucose transport assay
Epididymal and perirenal adipose tissues were removed from male Wistar rats weighing 160–200 g under anesthesia induced by an intraperitoneal injection of 100 mg/kg sodium pentobarbital, and isolated adipocytes were prepared by the collagenase method (15). As described previously (10), the glucose transport activity was assessed by measuring the rate of specific uptake of 3-O-methylglucose (3-O-MG) for 3 sec at 37°C, which was corrected for the non-specific uptake estimated using L-glucose. The uptake values were simply expressed as picomoles of 3-O-MG taken up specifically per 3 sec per 10 mg of adipocytes. Aliquots of pooled adipocytes were kept at 37°C for at least 15 min, and after incubations with the noted
agents for the indicated times, the glucose transport activity was determined as above. The insulin concentration (20 nM) employed in the present study provided the maximal insulin effect on 3-O-MG transport. Chromate (1 mM) did not change the equilibrium space of 3-O-MG in adipocytes.

**Statistical analyses**
All results are expressed as means±S.D. The two-tailed and one-tailed unpaired t-tests were applied as appropriate.

**RESULTS**

As shown in Fig. 1, sodium chromate significantly stimulated 3-O-MG uptake into adipocytes in a dose-dependent manner, and the stimulatory effect reached the maximum at around 300 μM. The maximal effect of chromate was approximately 66% of the maximal insulin effect that was obtained with 20 nM insulin. The chromate concentration achieving a half-maximal effect was estimated to be about 50 μM.

The time course of this stimulation is shown in Fig. 2. The 10-min stimulation produced the maximum effect, and hence this stimulation time was employed throughout the following experiments.

We next examined whether the effects of chromate and insulin were additive or not, and whether the effects were ATP-dependent or not. As shown in Table 1, the effect of the combination of 1 mM chromate and 20 nM insulin...
was similar to the effect of 20 nM insulin alone, showing that these two effects were not additive. As described previously (10, 11), adipocytes deprived of ATP using KCN were prepared for the latter purpose. No stimulatory effect of 1 mM chromate or 20 nM insulin was observed in such cells (Table 1), which indicated that these effects were completely ATP-dependent. This result was not due to non-specific KCN harm on the cells since the stimulatory effect was retained in adipocytes pre-stimulated with insulin before exposure to KCN (C in Table 1).

We next tested whether or not chromate (1 mM) directly stimulated glucose transport activity (the function of glucose transporters) recruited by insulin. Adipocytes pre-stimulated with 0.3 nM insulin were exposed to KCN and further incubated with 1 mM chromate or 20 nM insulin before measuring 3-O-MG uptake. As described previously (11, 16, 17), with this KCN intervention method, we could test effects of agents under the condition in which glucose transporters recruited by a submaximal concentration of insulin were fixed on the cell surface because their dynamic cycling was stopped with ATP depletion. As shown in Table 2, no stimulatory effect of 1 mM chromate or 20 nM insulin was seen under such a condition. No direct effect on the function of glucose transporters was eventually found.

We next examined what forms of the chromium compounds exerted the significant effect. As shown in Table 3, the oxide form containing a hexavalent chromium showed a large effect, while the compounds containing a trivalent chromium exhibited only a slight effect.

### Table 2. Effect of chromate on 3-O-MG uptake by adipocytes stimulated with 0.3 nM insulin before exposure to KCN

<table>
<thead>
<tr>
<th>Addition</th>
<th>3-O-MG uptake (pmol/3 sec/10 mg cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
</tr>
<tr>
<td>Buffer alone (Basal)</td>
<td>3.71 ± 0.22</td>
</tr>
<tr>
<td>1 mM Na₂Cr₂O₇</td>
<td>10.41 ± 1.11</td>
</tr>
<tr>
<td>20 nM Insulin</td>
<td>15.13 ± 0.56</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
</tr>
<tr>
<td>2 mM KCN alone</td>
<td>3.46 ± 0.32</td>
</tr>
<tr>
<td>20 nM Insulin + 2 mM KCN</td>
<td>14.86 ± 0.63</td>
</tr>
<tr>
<td>0.3 nM Insulin + 2 mM KCN + Buffer alone</td>
<td>11.89 ± 0.64</td>
</tr>
<tr>
<td>0.3 nM Insulin + 2 mM KCN + 1 mM Na₂Cr₂O₇</td>
<td>11.63 ± 0.42</td>
</tr>
<tr>
<td>0.3 nM Insulin + 2 mM KCN + 20 nM Insulin</td>
<td>11.85 ± 0.77</td>
</tr>
</tbody>
</table>

A: aliquots of adipocytes were incubated with the indicated agents for 10 min at 37°C, and 3-O-MG uptake was measured. B: aliquots of adipocytes that had been incubated with or without 0.3 nM or 20 nM insulin for 10 min at 37°C were exposed to 2 mM KCN for 5 min and further incubated with the indicated agents for 10 min prior to determining 3-O-MG uptake. Values are means ± S.D., n=6. 3-O-MG: 3-O-methylglucose.

### Table 3. Effects of various chromium compounds on 3-O-MG uptake

<table>
<thead>
<tr>
<th>Addition</th>
<th>3-O-MG uptake (pmol/3 sec/10 mg cells)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer alone (Basal)</td>
<td>3.97 ± 0.14</td>
<td>1.00</td>
</tr>
<tr>
<td>20 nM Insulin</td>
<td>16.18 ± 0.49</td>
<td>× 4.08</td>
</tr>
<tr>
<td>1 mM CrCl₂</td>
<td>4.26 ± 0.26</td>
<td>× 1.07</td>
</tr>
<tr>
<td>1 mM Cr₂O₇</td>
<td>4.37 ± 0.28*</td>
<td>× 1.10</td>
</tr>
<tr>
<td>1 mM CrO₃</td>
<td>10.77 ± 0.36**</td>
<td>× 2.71</td>
</tr>
<tr>
<td>1 mM Na₂Cr₂O₇</td>
<td>11.35 ± 0.79**</td>
<td>× 2.86</td>
</tr>
</tbody>
</table>

Aliquots of adipocytes were incubated with the indicated agents for 10 min at 37°C, and 3-O-MG uptake was measured. Values are means ± S.D., n=6. *P<0.05, **P<0.01 vs basal values. 3-O-MG: 3-O-methylglucose.

### DISCUSSION

In the present study, we found that chromate acted like an insulin-like agent on glucose transport. When the effect of chromate is compared with that of vanadate, molybdate and tungstate (10), chromate exhibits the strongest effect. This suggests that chromate may be a useful insulin-like agent when we study issues related to insulin actions in vitro.

Characterization of the action of chromate was carried out in the present study. The effects of 1 mM chromate and 20 nM insulin were not additive, and these effects were completely ATP-dependent as shown in Table 1 and described in the Results. These observations suggest that the chromate action is exerted through a mechanism analogous to that of insulin action.

To obtain further support for this idea, we examined whether or not chromate acted directly on the function of glucose transporters. As given in Table 2 and stated in the Results, chromate or insulin did not directly affect the transporter function. This result also supports the above idea. Although a major mechanism of insulin action is the translocation of glucose transporters from the intracellular pool to the plasma membrane (18 - 20), further studies are required to conclude whether this is the case with chromate action.

We determined that the CrO₄²⁻ moiety must be present in a chromium compound for it to have significant insulin-like activity (Table 3). Therefore, the necessary conditions are that a compound should contain a hexavalent chromium (not trivalent) and should presumably be an oxide form. Since VO₄³⁻, SeO₄²⁻, MoO₄²⁻ and WO₄²⁻ also show insulinomimetic actions (1 - 3, 5, 10 - 12), the oxide form may be a key to the action, although its mechanism has not been elucidated.

On the other hand, trivalent chromium is a trace element essential for life and is a component of glucose toler-
ance factor (13, 21). Tokuda et al. (22) demonstrated that 10 nM – 1 μM (concentrations expressed as chromium content) of glucose tolerance factor significantly stimulated glucose transport activity by rat adipocytes, while the same concentrations of Cr\(^{3+}\) or Cr\(^{6+}\) exhibited no effect, except that 1 pM Cr\(^{6+}\) only showed a small effect. Unlike trivalent chromium, hexavalent chromium is poisonous, and therefore it is difficult to develop hexavalent chromium compounds as a medicine for diabetes. Nevertheless, Cr\(^{4+}\) can be a useful tool for in vitro studies as a novel insulinomimetic agent.

REFERENCES


4 Ezaki O: IIb group metal ions (Zn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\)) stimulated glucose transport activity by post-insulin receptor kinase mechanism in rat adipocytes. J Biol Chem 264, 16118–16122 (1989)


