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, CrO₄²⁻ was responsible for the insulinomimetic action. These results indicate that the action of CrO₄²⁻ is exerted through a mechanism analogous to that of insulin action, and that CrO₄²⁻ is a novel and useful tool for studying issues involved in insulin actions.

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

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).

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

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**Fig. 1.** Chromate concentration-response curve for 3-O-MG uptake. Aliquots of adipocytes were incubated with the indicated concentrations of sodium chromate for 10 min at 37°C, and 3-O-MG uptake was measured. The closed circle denotes insulin-stimulated values obtained by substituting 20 nM insulin for chromate. The chromate concentration achieving a half-maximal effect was estimated to be about 50 μM. Values are means±S.D., n=6. **P<0.01 vs basal values. 3-O-MG: 3-O-methylglucose.

**Fig. 2.** Time course of the effect of 1 mM sodium chromate on 3-O-MG uptake. Aliquots of adipocytes were incubated with 1 mM Na2CrO4 for the designated times at 37°C, and 3-O-MG uptake was measured. The zero-time values indicate basal (unstimulated) values. The closed circle denotes insulin-stimulated values obtained by incubating cells with 20 nM insulin for 10 min before the measurement of 3-O-MG uptake. Values are means±S.D., n=6. 3-O-MG: 3-O-methylglucose.

**Table 1.** Effect of the combination of chromate and insulin on 3-O-MG uptake, and effect of ATP depletion on actions of chromate or insulin

| Addition                          | 3-O-MG uptake (pmol/3 sec/10 mg cells) |
|-----------------------------------|----------------------------------------|
| **A**                             |                                        |
| Buffer alone (Basal)              | 3.50±0.18                              |
| 1 mM Na2CrO4                     | 11.12±0.68                             |
| 20 nM Insulin                    | 15.78±0.58                             |
| Both                             | 15.28±0.51                             |
| **B**                             |                                        |
| 2 mM KCN alone                   | 3.18±0.16                              |
| KCN + 1 mM Na2CrO4               | 3.19±0.32                              |
| KCN + 20 nM Insulin              | 3.07±0.34                              |
| **C**                             |                                        |
| Insulin before KCN               | 15.04±1.03                             |

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 were preincubated with 2 mM KCN for 5 min at 37°C and further incubated with the indicated agents for 10 min prior to determining 3-O-MG uptake. C: aliquots of adipocytes were preincubated with 20 nM insulin for 10 min at 37°C and further incubated with 2 mM KCN for 15 min before measuring 3-O-MG uptake. Values are means±S.D., n=6. 3-O-MG: 3-O-methylglucose.
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 prestimulated 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 prestimulated 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

| Addition                  | 3-O-MG uptake (pmol/3 sec/10 mg cells) |
|---------------------------|----------------------------------------|
| A                         |                                        |
| Buffer alone (Basal)      | 3.71 ± 0.22                            |
| 1 mM Na₂CrO₄             | 10.41 ± 1.11                           |
| 20 nM Insulin            | 15.13 ± 0.56                           |
| B                         |                                        |
| 2 mM KCN alone           | 3.46 ± 0.32                            |
| 20 nM Insulin + 2 mM KCN | 14.86 ± 0.63                           |
| 0.3 nM Insulin + 2 mM KCN|                                        |
| + Buffer alone           | 11.89 ± 0.64                           |
| + 1 mM Na₂CrO₄           | 11.63 ± 0.42                           |
| + 20 nM Insulin          | 11.85 ± 0.77                           |

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

| Addition                  | 3-O-MG uptake (pmol/3 sec/10 mg cells) | Effect |
|---------------------------|----------------------------------------|--------|
| Buffer alone (Basal)      | 3.97 ± 0.14                            | 1.00   |
| 20 nM Insulin            | 16.18 ± 0.49                           | × 4.08 |
| 1 mM CrCl₃              | 4.26 ± 0.26                            | × 1.07 |
| 1 mM Cr₂O₇              | 4.37 ± 0.28*                           | × 1.10 |
| 1 mM CrO₄               | 10.77 ± 0.36**                         | × 2.71 |
| 1 mM Na₂CrO₄            | 11.35 ± 0.79**                         | × 2.86 |

A: 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 to 1 μM (concentrations expressed as chromium content) of glucose tolerance factor significantly stimulated glucose transport activity by rat adipocytes, while the same concentrations of Cr³⁺ or Cr⁶⁺ exhibited no effect, except that 1 pM Cr⁶⁺ 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, CrO₄²⁻ can be a useful tool for in vitro studies as a novel insulinomimetic agent.

REFERENCES

1 Tolman EL, Barris E, Burns M, Pansini A and Partridge R: Effects of vanadium on glucose metabolism in vitro. Life Sci 25, 1159-1164 (1979)
2 Dubyak GR and Kleinzeller A: The insulin-mimetic effects of vanadate in isolated rat adipocytes. Dissociation from effects of vanadate as a (Na⁺/K⁺) ATPase inhibitor. J Biol Chem 255, 5306-5312 (1980)
3 Tamura S, Brown TA, Whipple JH, Fujita-Yamaguchi Y, Dubler RE, Cheng K and Larner J: A novel mechanism for the insulin-like effect of vanadate on glycogen synthesis in rat adipocytes. J Biol Chem 259, 6650-6658 (1984)
4 Ezaki O: IIb group metal ions (Zn²⁺, Cd²⁺, Hg²⁺) stimulated glucose transport activity by post-insulin receptor kinase mechanism in rat adipocytes. J Biol Chem 264, 16118-16122 (1989)
5 Ezaki O: The insulin-like effects of selenate in rat adipocytes. J Biol Chem 265, 1124-1128 (1990)
6 Heyliger CE, Tahiliani AG and McNeill JH: Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science 227, 1474-1477 (1985)
7 Meyerovitch J, Farfel Z, Sack J and Shechter Y: Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats. J Biol Chem 262, 6658-6662 (1987)
8 Shisheva A, Gefel D and Shechter Y: Insulinlike effects of zinc ion in vitro and in vivo. Diabetes 41, 982-988 (1992)
9 McNeill JH, Delgatty HLM and Battell ML: Insulin-like effects of sodium selenate in streptozotocin-induced diabetic rats. Diabetes 40, 1675-1678 (1991)
10 Goto Y, Kida K, Ikeuchi M, Kaino Y and Matsuda H: Synergism in insulin-like effects of molybdate plus H₂O₂ or tungstate plus H₂O₂ on glucose transport by isolated rat adipocytes. Biochem Pharmacol 44, 174-177 (1992)
11 Goto Y, Kida K, Kaino Y, Ito T and Matsuda H: Actions of peroxovanadate or tungstate on glucose transport by isolated rat adipocytes. Acta Paediatr Jpn 36, 20-24 (1994)
12 Fillat C, Rodriguez-Gil JE and Guinovart JJ: Molybdate and tungstate act like vanadate on glucose metabolism in isolated hepatocytes. Biochem J 282, 659-663 (1992)
13 Schwarz K and Mertz W: Chromium III and the glucose tolerance factor. Arch Biochem Biophys 293, 224-230 (1992)
14 Goto Y, Kida K, Ikeuchi M, Kaino Y and Matsuda H: Evidence that polymyxin B is a glucose transport inhibitor. Biochem Pharmacol 42, 1399-1402 (1991)
15 Rodbell M: Metabolism of isolated fat cells. J Biol Chem 239, 375-380 (1964)
16 Goto Y, Sumida Y, Flanagan JE, Robinson FW, Simpson IA, Cushman SW and Kono T: Effects of fluorescein isothiocyanate on insulin actions in rat adipocytes. Arch Biochem Biophys 293, 224-230 (1992)
17 Goto Y, Kida K, Kaino Y, Ito T and Matsuda H: Inhibitory effect of amiloride on glucose transport in isolated rat adipocytes. Diabetes Res Clin Pract 20, 1-5 (1993)
18 Suzuki K and Kono T: Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. Proc Natl Acad Sci USA 77, 2542-2545 (1980)
19 Cushman SW and Wardzala LJ: Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. J Biol Chem 255, 4758-4762 (1980)
20 Kono T, Robinson FW, Blevins TL and Ezaki O: Evidence that translocation of the glucose transport activity is the major mechanism of insulin action on glucose transport in fat cells. J Biol Chem 257, 10942-10947 (1982)
21 Toepfer EW, Mertz W, Polansky MM, Roginski EE and Wolf WR: Preparation of chromium-containing material of glucose tolerance factor activity from brewer's yeast extracts. J Agric Food Chem 25, 162-166 (1977)
22 Tokuda M, Kashiwagi A, Wakamiya E, Oguni T, Mino M and Kagamiyama H: Glucose tolerance factor stimulates 3-O-methylglucose transport into isolated rat adipocytes. Biochem Biophys Res Commun 144, 1237-1242 (1987)