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Insulin-Like Actions of Nickel and Other Transition-Metal Ions in Rat Fat-Cells

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1. $NiCl_2$ (1–6mM) decreased adrenaline and glucagon-stimulated lipolysis in rat fat-cells, and also considerably stimulated [U-^{14}C]glucose incorporation into fat-cell lipids. 2. These insulin-like effects were also observed with CuCl, $CuCl_2$, $CoCl_2$ and (to a lesser extent) with $MnCl_2$. 3. $NiCl_2$ was less effective in mimicking insulin effects on [U-^{14}C]-fructose metabolism than on glucose utilization. 4. It is tentatively suggested that these transition-metal ions may mimic actions of insulin at the fat-cell plasma membrane which decrease lipolysis and stimulate glucose transport, but do not mimic certain other effects of the hormone on intracellular metabolic processes. 5. These results are discussed with reference to suggestions that redistributions of cellular Ca^{2+} are associated with insulin action in fat-cells.

Kissebah *et al.* (1974) and Siddle & Hales (1974) have proposed a role for Ca^{2+} in the regulation of adipose-tissue lipolysis. In addition, it has been suggested that actions of insulin other than the antilipolytic effect may be mediated by, or associated with, intracellular redistributions of Ca^{2+} in adipose tissue (Clausen *et al.*, 1974; Hope-Gill *et al.*, 1974; Severson *et al.*, 1974). Ni^{2+} has been reported to antagonize actions of Ca^{2+} in insulin regulation of sugar transport in diaphragm (Bihler, 1972), in excitation-contraction coupling (Kaufman & Fleckenstein, 1965; Kleinfeld & Stein, 1968; Kohlhardt *et al.*, 1973) and in presynaptic neurotransmitter release (Benoit & Mambrini, 1970), and shows a certain amount of antagonism to Ca^{2+} in stimulus-secretion coupling (Dormer *et al.*, 1974). It was therefore decided to investigate the effect of Ni^{2+} on fat-cell lipolysis in the expectation that it could either block the antilipolytic action of insulin or even itself be lipolytic. The converse, however, was found. Ni^{2+} and some other transition-metal ions inhibited hormone-stimulated lipolysis. These ions were found to mimic insulin action further in that they also considerably stimulated [^{14}C]glucose incorporation into fat-cell lipids. It is suggested that Ni^{2+} may be capable of mimicking some fundamental action of insulin in the fat-cell, presumably at the plasma membrane.

Materials and Methods

Chemicals

Enzymes (except for collagenase), nucleotides and sodium pyruvate were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Adrenaline, glucagon (from a mixture of ox and pig pancreas), 3′:5′-cyclic AMP (sodium salt), 6-*N*,2′-*O*-dibutyryl 3′:5′-cyclic AMP (sodium salt), theophylline (1,3-dimethylxanthine), D-fructose and calf thymus DNA were obtained from Sigma (London) Chemical Co., London S.W.6, U.K. Collagenase (type I, from *Clostridium histolyticum*) was from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Bovine serum albumin (fraction V; also from Sigma) was treated to remove fatty acids as described by Saggerson (1972). Sodium palmitate from Nu Chek Prep, Elysian, MN, U.S.A. was associated with fatty acid-poor albumin as described by Evans & Mueller (1963). Radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. Bovine insulin (6×recrystallized) was obtained from Boots Pure Drug Co. Ltd., Nottingham, U.K. 2,5-Bis-(5-t-butylbenzoxazol-2-yl)thiophen was from CIBA (A.R.L.) Ltd., Duxford, Cambridge, U.K.

Animals

These were male Sprague–Dawley rats weighing 150–200g, which were maintained on cube diet 41B (Bruce & Parkes, 1949). Water was supplied at all times.

Techniques with fat-cells

Preparation. Isolated fat-cells were prepared by the method of Rodbell (1964) as previously described (Saggerson & Tomassi, 1971).

Incubation. Portions of fat-cells were incubated in 4ml of Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) previously gassed with O_2+CO_2 (95:5) in shaken 25ml silicone-treated Erlenmeyer

flasks as described by Saggerson & Tomassi (1971). Fatty acid-poor albumin was present at a concentration of 30mg/ml in experiments in which lipolysis was measured. In all other experiments albumin was used at a concentration of 17mg/ml. When additions of various metal chlorides were made to incubation media it was observed that addition of Ni^{2+}, Co^{2+} and Mn^{2+} (6mM) or of Ba^{2+} (2mM) did not result in the visible formation or precipitation of insoluble salts during incubations. Addition of Cu^{+} (6mM) and Cu^{2+} (2mM) resulted in an initial precipitate which immediately redissolved on shaking. No re-precipitation was observed during incubation with the above metal ions. Addition of Cr^{3+} and Fe^{3+} (2mM) resulted in some precipitation, which did not redissolve during incubation. ^{14}C-labelled substrates, where appropriate, were present at 0.25μCi/ml. In one series of experiments (see Fig. 4), in which fat-cells were withdrawn at frequent times from the incubation, it was not convenient to incubate the cells in sealed shaken flasks. The cells were therefore incubated in a stirred, water-jacketed chamber as described by Sooranna & Saggerson (1975). The quantity of fat-cells present in incubations is indicated in the legends to the individual Figures and Tables.

Measurement of incorporation of radioactivity into metabolic products. After incubation, lipids from fat-cells were extracted into hexane (Saggerson & Tomassi, 1971). These extracts were then analysed for incorporation of ^{14}C into glyceride fatty acids and glyceride glycerol as described by Saggerson & Greenbaum (1970) and Saggerson & Tomassi (1971).

Measurement of glycerol. Portions of incubation media containing fat-cells were deproteinized (Saggerson, 1972) and assayed for glycerol as described by Garland & Randle (1962).

Measurement of fat-cell DNA. This was as described by Saggerson (1972).

Expression of results

Units for incorporation of substrates into lipids or for production of glycerol are indicated in the individual legends to Figures and Tables. In all experiments one determination represents a different fat-cell preparation. Where presented, statistical significance of results was determined by Student's *t* test.

Results and Discussion

Effects of metal ions on lipolysis

Fig. 1 shows that $NiCl_2$ antagonized the lipolytic effects of adrenaline (1μM) and of glucagon (2μg/ml). In the presence of either hormone, lipolysis was decreased to basal values at $NiCl_2$ concentrations above 3mM. The effect of 2mM-$NiCl_2$, which partially abolished hormone-stimulated lipolysis, was then tested over a range of adrenaline concentrations (Fig. 2). Ni^{2+} decreased lipolysis at all adrenaline

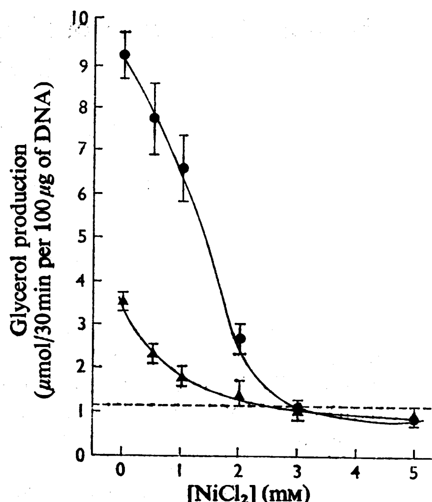

Fig. 1. *Effect of Ni^{2+} on adrenaline- and glucagon-induced lipolysis in fat-cells*

Fat-cells were incubated for 30min in Krebs–Ringer bicarbonate buffer containing albumin (30mg/ml), 5mM-glucose, the indicated concentration of $NiCl_2$ and either adrenaline (1.0μM) or glucagon (2.0μg/ml). The results are the means of three determinations. The vertical bars represent S.E.M. and the dashed line indicates basal lipolysis. The mean fat-cell DNA content was 2.1μg/ml of incubation medium. ●, With adrenaline; ▲, with glucagon.

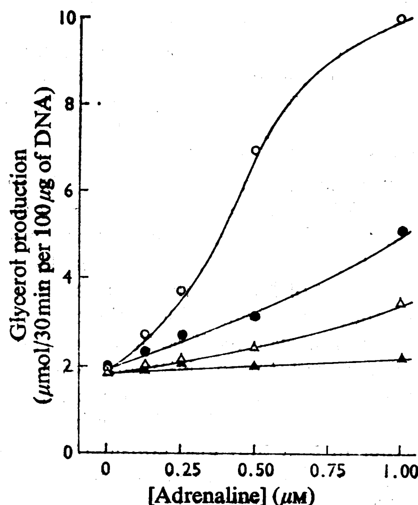

Fig. 2. *Effects of Ni^{2+} and insulin on fat-cell lipolysis at various adrenaline concentrations*

Fat-cells were incubated for 30min in Krebs–Ringer bicarbonate buffer containing albumin (30mg/ml), 5mM-glucose, the indicated concentration of adrenaline, and where appropriate, $NiCl_2$ (2mM) or insulin (20munits/ml). The results are taken from a single experiment in which the fat-cell DNA was 2.0μg/ml of incubation medium. ○, No further additions; ●, with Ni^{2+}; △, with insulin; ▲, with Ni^{2+}+insulin.

concentrations tested, but had no effect on basal lipolysis; 2mM-$NiCl_2$ also further decreased adrenaline-stimulated lipolysis in the presence of 20munits of insulin/ml, which is a saturating concentration of this hormone. $NiCl_2$ therefore showed insulin-like actions, although the antilipolytic effect of $NiCl_2$ was at least partially additive to that of insulin. Table 1 shows that some other metal ions at concentrations of 2mM were antagonistic to adrenaline-stimulated lipolysis. Co^{2+}, Fe^{3+}, Cu^{+} and Cu^{2+} were effective in this respect, whereas Ba^{2+} and Cr^{3+} had no significant effects. Co^{2+}, Cu^{+} and Cu^{2+} also decreased glucagon-stimulated lipolysis (Table 2). However, 2mM-$MnCl_2$ had no effect on glucagon-stimulated lipolysis. This was perhaps an unexpected result in view of other insulin-like actions of Mn^{2+} on adipose tissue [see Fig. 3 and Baquer *et al.* (1975)]. The concentration of $CaCl_2$ in the incubation medium did not greatly influence the effect of $NiCl_2$ on adrenaline-stimulated lipolysis. This was shown in a single experiment in which fat-pads were treated with collagenase in the normal manner in Krebs–Ringer bicarbonate buffer containing 1.27mM-$CaCl_2$. After the collagenase digestion the fat-cells were washed three times in Ca^{2+}-free medium and then incubated for 30min with 1μM-adrenaline and various concentrations of $CaCl_2$ (0.25–3.0mM). The effect of 2mM-$NiCl_2$ in decreasing lipolysis was approximately the same at all tested concentrations of Ca^{2+}, i.e. at 0.25, 0.5, 1.0, 1.5, 2.0 and 3.0mM-Ca^{2+}, lipolysis in the presence of 2mM-Ni^{2+} was 73, 60, 52, 64, 60 and 56% respectively compared with lipolysis in the absence of Ni^{2+}. $NiCl_2$ also mimicked insulin action in decreasing lipolysis induced by methylxanthines (Hepp *et al.*, 1969), i.e. lipolysis, which was increased to 31 times the basal value by 0.5mM-theophylline, was decreased 58% by 2mM-$NiCl_2$. $NiCl_2$, however, exceeded the effects of insulin on lipolysis induced by 6-*N*,2′-*O*-dibutyryl 3′:5′-cyclic AMP or 3′:5′-cyclic AMP. Insulin has been reported to be unable to block lipolysis induced by these agents (Hepp *et al.*, 1969), but 2mM-$NiCl_2$ decreased lipolysis induced by 1mM-dibutyryl cyclic AMP (970% of basal) and 1mM-cyclic AMP (195% of basal) by 16 and 44% respectively (results not shown, two determinations). These effects may be compared with the 60–70% decreases in adrenaline or glucagon-induced lipolysis caused by 2mM-$NiCl_2$ (Fig. 1).

Table 1. *Effect of metal ions on adrenaline-induced lipolysis in fat-cells*

Fat-cells were incubated for 30min in Krebs–Ringer bicarbonate buffer containing albumin (30mg/ml), 5mM-glucose, the indicated metal chloride (2mM in every case) and, where appropriate, adrenaline (1.0μM). The results are means±S.E.M. of three determinations. The mean fat-cell DNA content was 2.0μg/ml of incubation medium. *, ** represent $P<0.01$ and <0.001 respectively for cells incubated with metal ions compared with the adrenaline-treated control.

Additions to incubation medium	Glycerol release (μmol/30min per 100μg of DNA)
None	1.69±0.20
Adrenaline	9.05±0.25
Adrenaline+$NiCl_2$	4.62±0.85*
+$CoCl_2$	2.22±0.42**
+$BaCl_2$	8.83±0.30
+$FeCl_3$	2.93±0.24**
+$CrCl_3$	7.72±0.27
+CuCl	1.48±0.48**
+$CuCl_2$	1.11±0.26**

Table 2. *Effect of metal ions on glucagon-induced lipolysis in fat-cells*

Fat-cells were incubated for 30min in Krebs–Ringer bicarbonate buffer containing albumin (30mg/ml), 5mM-glucose, the indicated metal chloride (2mM in every case) and, where appropriate, glucagon (2μg/ml). The results are means±S.E.M. of three determinations. The mean fat-cell DNA content was 1.9μg/ml of incubation medium. *, ** represent $P<0.02$ and <0.01 respectively for cells incubated with metal ions compared with the glucagon-treated control.

Additions to incubation medium	Glycerol release (μmol/30min per 100μg of DNA)
None	1.27±0.15
Glucagon	3.96±0.60
Glucagon+$NiCl_2$	1.13±0.05**
+$CoCl_2$	1.70±0.04*
+$MnCl_2$	4.02±0.32
+CuCl	0.96±0.03**
+$CuCl_2$	0.75±0.10**

Effects of metal ions on lipogenesis

Ni^{2+} and some other metal ions also showed insulin-like actions in promoting glucose incorporation into both the fatty acid and glycerol moieties of fat-cell glycerides (Table 3). Ni^{2+}, Cu^{+} and Cu^{2+} showed the same relative potencies in promoting lipogenesis as in their antilipolytic actions. However, 2mM-$CoCl_2$, which had a similar or even greater antilipolytic effect than 2mM-$NiCl_2$ (Tables 1 and 2), was relatively ineffective in promoting lipogenesis. In other experiments (two determinations, results not shown), 2mM-$BaCl_2$ had no effect on lipogenesis, whereas $CrCl_3$ and $FeCl_3$ inhibited lipogenesis by 20–30%. Owing to the precipitation of insoluble salts these small effects of Cr^{3+} and Fe^{3+} could not be interpreted, and no further investigations were made. When palmitate was also included in the incubation medium to provide a source of substrate for glyceride synthesis, the effects of metal ions (and insulin) on

Table 3. *Effect of metal ions on fat-cell lipogenesis from glucose*

Fat-cells were incubated for 1 h in Krebs–Ringer bicarbonate buffer containing 17 mg of albumin/ml, 5 mM-[U-^{14}C]glucose and, where indicated, metal chloride (2 mM in every case), sodium palmitate (0.5 mM) or insulin (20 munits/ml). The results, which are expressed as μg-atoms of carbon/h per 100 μg of DNA, are means ± S.E.M. of three determinations for Expt. 1 (mean fat-cell DNA content was 3.4 μg/ml of incubation medium) and of four determinations for Expt. 2 (mean fat-cell DNA was 2.9 μg/ml). *, **, *** represent $P<0.05$, 0.01, 0.001 respectively for comparison of insulin- or metal ion-treated cells with the appropriate insulin- or metal ion-free controls.

Expt. no.	Additions to incubation medium	[U-^{14}C]Glucose incorporation into: Fatty acids	Glyceride glycerol
1	None	1.20±0.07	1.23±0.03
	$NiCl_2$	3.79±0.29***	1.33±0.06
	$CoCl_2$	1.89±0.26	1.11±0.13
	CuCl	5.51±0.84**	1.60±0.23
	$CuCl_2$	7.83±1.41**	2.12±0.07***
	Insulin	10.32±0.59***	2.70±0.21**
	Sodium palmitate	1.03±0.21	3.73±0.30
	Sodium palmitate+$NiCl_2$	4.66±1.06*	12.11±1.29**
	+$CoCl_2$	1.67±0.18	6.12±0.37**
	+CuCl	6.87±0.73**	12.82±0.50***
	+$CuCl_2$	5.14±1.37*	11.32±1.88*
	+insulin	13.39±0.87***	17.22±1.34***
2	None	1.19±0.06	1.47±0.24
	Insulin	10.49±0.45	2.95±0.29
	Insulin+$NiCl_2$	9.47±0.49	2.50±0.41
	+$CoCl_2$	9.15±0.37	3.11±0.83
	+CuCl	13.40±1.03*	3.65±0.81
	+$CuCl_2$	9.63±1.88	3.08±0.67

glyceride glycerol formation were able to be more fully expressed. When lipogenesis was stimulated by insulin, addition of 2 mM-$NiCl_2$, -$CoCl_2$ or -$CuCl_2$ resulted in no further increase in lipogenesis. There was therefore no evidence that Ni^{2+} and insulin may exert additive effects on lipogenesis as was found for lipolysis. However, 2 mM-CuCl gave a small stimulation of lipogenesis in addition to that elicited by insulin.

When 5 mM-[U-^{14}C]fructose replaced glucose as substrate it was found that $NiCl_2$ (3 mM) did not have any appreciable effect on lipogenesis, although lipogenesis from fructose was sensitive to insulin, i.e. insulin (20 munits/ml) increased fructose incorporation into fatty acids from 3.21±0.54 to 6.92±0.43 μg-atoms of carbon/h per 100 μg of DNA. In the presence of 3 mM-$NiCl_2$ the corresponding value was 3.23±0.17 μg-atoms of carbon/h per 100 μg of DNA (four determinations). Similarly, in the presence of 0.5 mM-palmitate, fructose incorporation into fatty acids was increased from 1.35±0.10 to 2.79±0.10 μg-atoms of carbon/h per 100 μg of DNA by insulin, whereas $NiCl_2$ (3 mM) only increased the value to 1.43±0.09. In the same experiment the values for glyceride glycerol formation were: control, 4.63±0.13; with insulin, 7.61±0.34; with Ni^{2+}, 5.18±0.43 μg-atoms of carbon/h per 100 μg of DNA (four determinations). As with the antilipolytic effects, the lipogenic effect of $NiCl_2$ was not affected by the concentration of Ca^{2+} in the incubation. This was shown in an experiment in which fat-cells previously washed three times in Ca^{2+}-free medium were incubated with [U-^{14}C]glucose and 0, 0.8, 2.0 or 5.0 mM-$NiCl_2$. In each case the $CaCl_2$ concentration in the medium was varied from 0.25 to 4.0 mM without any significant alteration in the stimulation of lipogenesis produced by a given concentration of Ni^{2+}.

The experiment shown in Fig. 3 was carried out to investigate the concentration-dependence of the interaction of Ni^{2+} with lipogenesis from glucose and fructose. In addition, the effects of Cu^{+} and Mn^{2+} were further investigated. These ions appeared to be of some interest in view of the small additive effect of 2 mM-CuCl and insulin on lipogenesis (Table 3) and in view of the reported additive effects of 1 mM-$MnSO_4$ and insulin on [1-^{14}C]glucose metabolism in incubated fat-pads (Baquer *et al.*, 1975). At concentrations of 3 and 6 mM, $NiCl_2$ and CuCl considerably stimulated glucose incorporation into fatty acids. The stimulation by $MnCl_2$ was less (Fig. 3*a*). In the presence of insulin, however, Ni^{2+} and Mn^{2+} decreased fatty acid synthesis and any stimulation by Cu^{+} was not apparent at concentrations above 3 mM (Fig. 3*c*). Ni^{2+} and Cu^{+} also influenced glyceride glycerol formation, increasing the rate almost to that found with insulin (Fig. 3*b*). The virtual absence of effects of

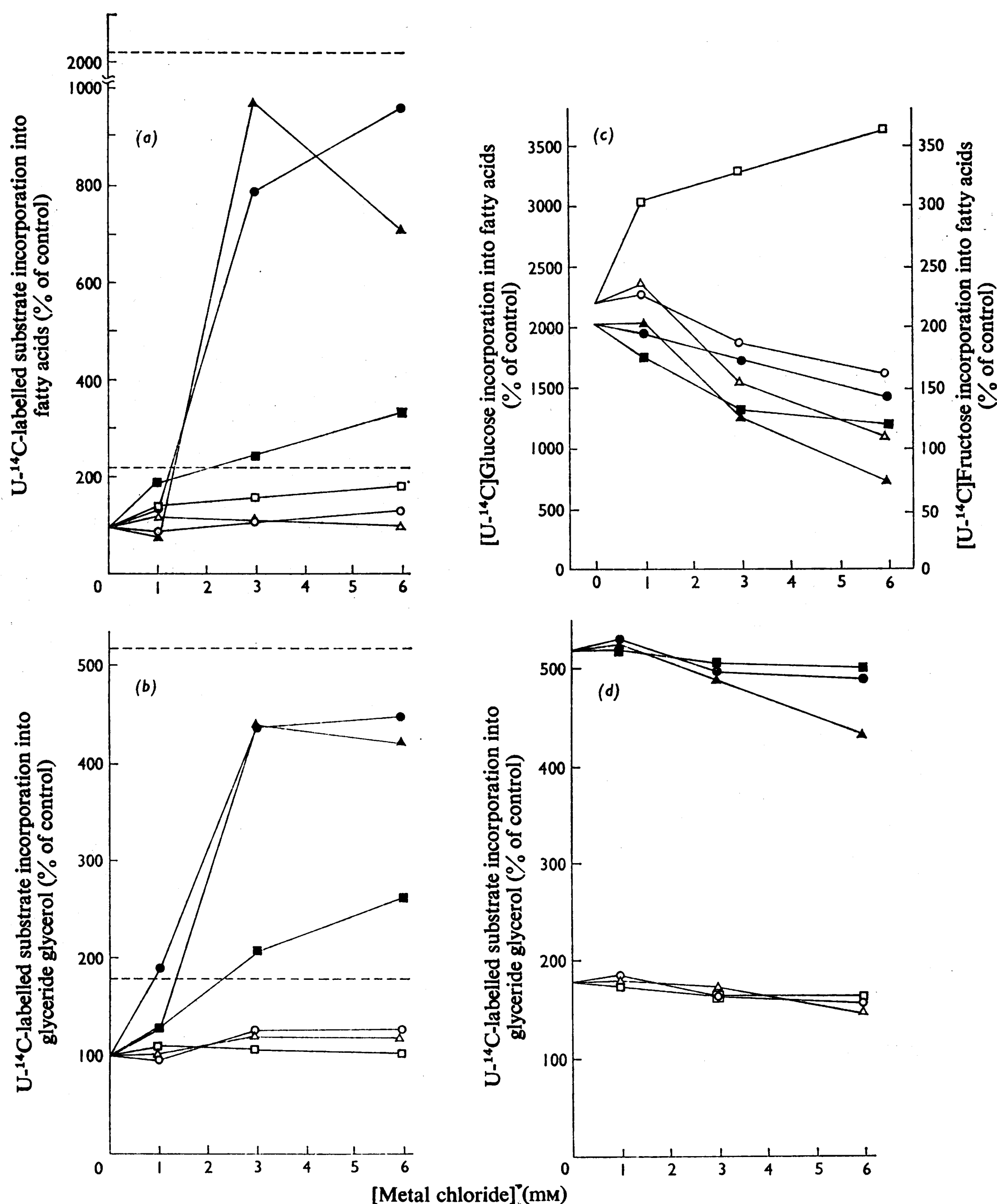

Fig. 3. *Effects of Ni^{2+}, Cu^{+} and Mn^{2+} on fat-cell lipogenesis from glucose and fructose*

Fat-cells were incubated for 1h in Krebs–Ringer bicarbonate buffer containing albumin (17mg/ml), 0.5mM-sodium palmitate, the indicated concentration of metal chloride, insulin (20munits/ml) where appropriate, and either 5mM-[U-^{14}C]-glucose or 5mM-[U-^{14}C]fructose. The results are the means of two determinations and are expressed as percentages of the control values in the absence of metal chlorides or insulin. The mean fat-cell DNA content was 1.9μg/ml of incubation medium. ●, Glucose+Ni^{2+}; ▲, glucose+Cu^{+}; ■, glucose+Mn^{2+}; ○, fructose+Ni^{2+}; △, fructose+Cu^{+}; □, fructose+Mn^{2+}. (*a*) Fatty acid synthesis: the upper dashed line indicates the percentage effect of insulin on glucose incorporation; the lower line indicates the effect of insulin on fructose incorporation. (*b*) Glyceride glycerol synthesis: the upper and lower dashed lines refer to the effect of insulin on glucose and fructose incorporation as in (*a*). (*c*) Fatty acid synthesis in the presence of insulin. (*d*) Glyceride glycerol synthesis in the presence of insulin.

Ni^{2+}, Cu^{+} or Mn^{2+} on glucose incorporation into glyceride glycerol in the presence of insulin (Fig. 3*d*) suggested that the inhibitory effects of these ions on fatty acid synthesis (Fig. 3*c*) arose from interactions with the lipogenic pathway distal to triose phosphate formation and were unlikely to be due to interference with the interaction of the hormone with plasma-membrane receptors or some other closely related event. In the absence of insulin, therefore, it is apparent that the concentration profiles of metal-ion action on fatty acid synthesis from glucose consist of a stimulatory effect and the inhibitory effect that is observed at higher concentrations of metal ion or when fatty acid synthesis is already maximally stimulated by insulin. In this respect it is pertinent that Ni^{2+} inhibits pyruvate dehydrogenase phosphate phosphatase in fat-cell mitochondria (Severson *et al.*, 1974). However, Mn^{2+} may be expected to increase the activity of pyruvate dehydrogenase phosphate phosphatase (Denton *et al.*, 1975; Hucho *et al.*, 1972). The effects of Cu^{+} on pyruvate dehydrogenase phosphate phosphatase activity do not appear to have been reported, although Denton *et al.* (1975) have reported Co^{2+} and Cu^{2+} as being ineffective. At present the nature of the inhibitory effects of the various metal ions is unclear. The effects may be multiple and may differ according to the ion in question. The lack of an inhibitory effect of quite high concentrations of $NiCl_2$ on neutral lipid synthesis (as measured by incorporation of glucose into glyceride glycerol) was noteworthy, since Jamdar & Fallon (1973*a,b*) found that 1mM-$NiCl_2$ in adipose-tissue extracts almost completely abolished phosphatidate phosphatase activity with membrane-bound substrate but had no effect on the activity when the substrate was in aqueous solution.

When [^{14}C]fructose was used as substrate an appreciable effect of Mn^{2+} on fatty acid synthesis was observed (Fig. 3*a*), but effects of Ni^{2+} and Cu^{+} on fatty acid synthesis, and of Ni^{2+}, Cu^{+} and Mn^{2+} on glyceride synthesis, were small. This relative lack of effect of the metal ions on fructose utilization can best be discerned if the stimulation of a particular parameter due to the action of a metal ion is expressed as a percentage of the stimulation of the same parameter by insulin (in the absence of metal ion). At 3mM concentrations of metal chloride, Ni^{2+} and Cu^{+} mimicked the insulin effect on fatty acid synthesis from glucose by 36% and 45% respectively, but only mimicked the effect of insulin on fatty acid synthesis from fructose by 8% and 15% respectively. Similarly, 3mM-$NiCl_2$, -CuCl and -$MnCl_2$ mimicked the insulin stimulation of glucose incorporation into glyceride glycerol by 80, 80 and 26% respectively, but only mimicked the insulin effect on glyceride glycerol formation from fructose by 31, 28 and 9% respectively. These effects almost certainly have most meaning when applied to glyceride glycerol formation, since this occurs 'early' in the pathways of carbohydrate utilization. Fatty acid synthesis, however, is a more complex process, the effects of metal ions are more complex, and under the incubation conditions used some effects of insulin (and presumably of other agents) may be secondary to effects on glyceride synthesis (Sooranna & Saggerson, 1975). In rat adipose tissue the intracellular pathways of fructose and glucose metabolism are believed to be the same, although the utilization of these two sugars probably differs in the nature of their transport into the cell (Froesch & Ginsberg, 1962; Fain, 1964) and in the relative insensitivity of fructose uptake to insulin (Coore *et al.*, 1971). Some of the stimulatory actions of insulin on fructose utilization for fatty acid synthesis and possibly in glyceride synthesis cannot be explained fully on the basis of hormonal stimulation of plasma-membrane transport of the sugar (Coore *et al.*, 1971; Sooranna & Saggerson, 1975). The effects of Ni^{2+} and Cu^{+} are therefore not inconsistent with an insulin-mimicking action on the fat-cell membrane resulting in a relatively large stimulation of glucose utilization but a relatively small effect on fructose metabolism, leaving a significant proportion of the effect of insulin on fructose utilization to be explained by some other mechanism. The effects of Mn^{2+} on glyceride glycerol formation may also possibly be explained in this manner. However, the effects of Mn^{2+} on fatty acid synthesis were anomalous in that the insulin-dependent and Mn^{2+}-dependent stimulations of fructose incorporation were additive (insulin approximately doubled fatty acid synthesis at all Mn^{2+} concentrations tested), whereas, unlike Baquer *et al.* (1975), we did not observe additive effects of insulin and Mn^{2+} on fatty acid synthesis from glucose. These observations are unexplained.

Insulin has been shown to stimulate the incorporation of pyruvate into adipose-tissue fatty acids when low concentrations of this metabolite are supplied as sole substrate (Halperin, 1971; Sooranna & Saggerson, 1975). It was observed, however, that Ni^{2+} could not mimic this action of insulin. When fat-cells were incubated for 1h with 0.5mM-[2-^{14}C]-pyruvate, the incorporation of ^{14}C into fatty acids was decreased by 8, 27 and 28% by 1, 3 and 6mM-$NiCl_2$ respectively (results not shown).

The experiment shown in Fig. 4 demonstrates the time-dependence of the effect of a relatively high $NiCl_2$ concentration on glucose incorporation into glyceride glycerol (palmitate was present). Unlike the effect of insulin, which was extremely rapid, the effect of Ni^{2+} was only fully expressed after a delay of about 5min. The non-linearity of the time-courses at later times is due to depletion of palmitate in the incubation medium.

General discussion

A wide range of treatments show insulin-like

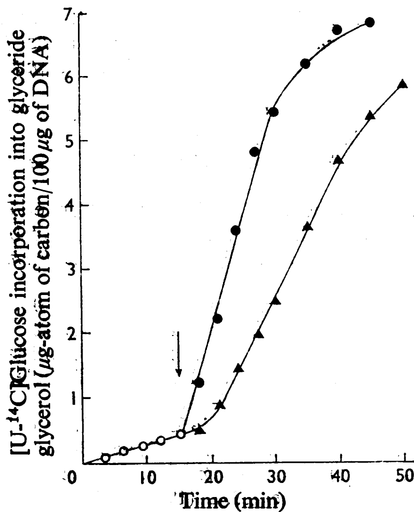

Fig. 4. *Time-course of stimulation of glyceride synthesis in fat-cells by insulin or* $NiCl_2$

Fat-cells were incubated in two paralleled stirred chambers in 12ml of Krebs–Ringer bicarbonate buffer containing albumin (17mg/ml), 0.55mM-sodium palmitate and 2mM-[U-^{14}C]glucose. Samples (0.5ml) were withdrawn for analysis at the indicated times. After 15min (indicated by the arrow), insulin (20munits/ml) or $NiCl_2$ (6mM) was added. The results are the means of two experiments. The mean fat-cell DNA content was 7.9µg/ml of incubation medium. ○, No additions; ●, with insulin; ▲, with $NiCl_2$.

actions in adipose tissue. The property of the hormone both to increase glucose uptake and metabolism and to decrease hormone-stimulated lipolysis has been mimicked by the following: ouabain and K^+ depletion (Ho & Jeanrenaud, 1967; Ho *et al.*, 1967; Letarte *et al.*, 1969); nicotinate (Lee *et al.*, 1961; Carlson & Bally, 1965; Ho & Jeanrenaud, 1967); phospholipase C action (Blecher, 1965; Rodbell, 1966; Rodbell & Jones, 1966); exposure to low concentrations of chymotrypsin, Pronase, subtilopeptidase A or trypsin (Kuo *et al.*, 1966, 1967*a*; Kuo, 1968*a*); *p*-chloromercuribenzenesulphonate (Minemura & Crofford, 1969); thiols (Lavis & Williams, 1970); RNA (Dole, 1961, 1962); arsenite (Kuo *et al.*, 1967*b*); polyene antibiotics (Kuo, 1968*b*); naphthoquinones (Fain, 1971); and procaine (Kissebah *et al.*, 1974; Hope-Gill *et al.*, 1974). In addition, insulin action on glucose utilization alone may be mimicked by phospholipase A action and lysophosphatides (Blecher, 1966, 1967), mild neuraminidase action (Cuatrecasas & Illiano, 1971) and protamine (Ball & Jungas, 1964). To this extensive list we now add Ni^{2+} and also, although the main emphasis of this study was on Ni^{2+}, other transition-metal ions. In this respect it is noteworthy that Bihler (1972) has reported that La^{3+}, Ni^{2+}, Zn^{2+}, Mn^{2+} and Co^{2+} can stimulate sugar transport in rat diaphragm, although this effect was only noted in the absence of Ca^{2+}. It has also been reported that $CdCl_2$ stimulates glucose utilization by adipose tissue *in vitro* [J. P. Flatt & F. G. Ball, unpublished work; reported by Ball & Jungas (1964)].

Although Ni^{2+} appeared to mimic insulin both in its effects on glucose utilization and in its antilipolytic action, it may be questioned as to how complete is the mimicry and whether the effects on lipolysis and glucose utilization are connected. Glucose incorporation into glyceride glycerol in the presence of an exogenous fatty acid substrate is a convenient and satisfactory parameter to measure when quantifying insulin effects on glucose transport in fat-cells. Ni^{2+} mimicked the effect of insulin on this process most appreciably. However, Ni^{2+} was far less effective in mimicking insulin effects on fructose metabolism and showed no insulin-like actions on pyruvate utilization. The latter observation may, however, result partly from interference of Ni^{2+} with the regulation of pyruvate dehydrogenase (Severson *et al.*, 1974). We advance the tentative suggestion that Ni^{2+} mimics the action of insulin at the fat-cell plasma membrane that promotes sugar transport but does not mimic those effects that are dependent on a postulated insulin 'second messenger' (Coore *et al.*, 1971). With lipolysis, Ni^{2+} appeared to exert actions in excess of those normally associated with insulin. Although a Ni^{2+}, Co^{2+}- or Mn^{2+}-activated cyclic nucleotide phosphodiesterase activity has been observed in rat liver (Campbell & Pearce, 1973) and may presumably be found in other tissues including adipose, this enzyme has the wrong specificity to be effective in decreasing 3':5'-cyclic AMP concentrations. The site(s) of action of Ni^{2+} on lipolysis is therefore unclear at present. A linkage between the metal-ion effects on glucose utilization and lipolysis is implied by the similar relative potencies of Ni^{2+}, Cu^+ and Cu^{2+}. This correlation, however, breaks down when Co^{2+} is considered, and it is therefore not possible at present to infer a close connexion of the lipogenic and antilipolytic effects.

There have been suggestions that certain actions of insulin in the fat-cell may be mediated through changes in the cellular distribution of Ca^{2+} (Kissebah *et al.*, 1974; Clausen *et al.*, 1974; Severson *et al.*, 1974; Siddle & Hales, 1974). The way in which Ca^{2+} redistribution is brought about is at present extremely unclear. Nevertheless, it is noteworthy that in other experimental systems Ni^{2+}, Co^{2+}, Mn^{2+} or Cu^{2+} may antagonize actions of Ca^{2+} (Kaufman & Fleckenstein, 1965; Kleinfeld & Stein, 1968; Benoit & Mambrini, 1970; Bihler, 1972; Meiri & Rahaminoff, 1972; Kohlhardt *et al.*, 1973; McNamara *et al.*, 1974), inhibit Na^+/Ca^{2+} exchange (Baker *et al.*, 1969; Clausen, 1970; Bihler, 1972) and in some cases substitute for Ca^{2+} (Kaufman & Fleckenstein, 1965;

Bihler, 1972). All of these possibilities could at present be advanced in speculation concerning the insulin-like actions of Ni^{2+} in fat-cells. Another possibility that may be considered is that Ni^{2+} may mimic insulin action through displacement or mobilization of Ca^{2+} from membranous storage or binding sites (Clausen *et al.*, 1974; Hales *et al.*, 1974). Insulin is known to decrease Ca^{2+} binding to artificial membranes (Kafka & Pak, 1969) and to preparations of liver plasma membrane (Marinetti *et al.*, 1972). Interaction of Ni^{2+} with membrane components could presumably produce the same effect. Present theories concerning the involvement of Ca^{2+} in insulin action must take note of the observation that insulin action on glucose utilization in fat-cells is not influenced by the concentration of Ca^{2+} in the incubation medium (Letarte & Renold, 1969), suggesting that any Ca^{2+} pool influenced by insulin should be distinct from the extracellular pool. A similar lack of dependence on the extracellular calcium concentration was noted for the effects of Ni^{2+} reported in the present study.

Although no definite explanations can at present be advanced for the effects of these transition-metal ions reported here, we consider that they merit further study and may possibly provide physical probes for the elucidation of insulin action.

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