Catecholamine-induced insulin resistance of glucose transport in isolated rat adipocytes

Diethelm M. KIRSCH,*† Michael BAUMGARTEN,* Thomas DEUFEL,* Franz RINNINGER,*
Wolfgang KEMMLER* and Hans U. HÄRING†

*Institut für Diabetesforschung, Kölner Platz 1, 8000 München 40, Federal Republic of Germany, and

†Elliot P. Joslin Research Laboratory, Boston, MA, U.S.A.

(Received 11 July 1983/Accepted 15 September 1983)

The effects of pre-incubation with isoprenaline and noradrenaline on insulin binding and insulin stimulation of D-glucose transport in isolated rat adipocytes are reported. (1) Pre-incubation of the cells with isoprenaline (0.1–10 μ M) in Krebs–Ringer–Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer (30 min, 37°C) at D-glucose concentrations of 16 mM, in which normal ATP levels were maintained, caused a rightward-shift in sensitivity of D-glucose transport to insulin stimulation by 50% and a decrease in maximal responsiveness by 30%. (2) [A14-¹²⁵I]insulin binding was reduced significantly by 35% at insulin concentrations less than 100 μ -units/ml and Scatchard analysis showed that this consisted mainly of a decrease in high-affinity binding. (3) Pre-incubation with catecholamines under the same conditions but at low glucose concentrations (0–5 mM) caused a fall in intracellular ATP levels of 65 and 45% respectively. (4) The fall in ATP additionally lowered insulin binding by 50% at all insulin concentrations and a parallel shift of the binding curves in the Scatchard plot showed that this was due to a decrease in the number of receptors. (5) At low and high ATP concentrations the insulin stimulation of D-glucose transport was inhibited to a similar extent. (6) Pre-incubation with catecholamines thus inhibited insulin stimulation of D-glucose transport in rat adipocytes mainly by a decrease in high-affinity binding of insulin, which was not mediated by low ATP levels. This mechanism may play a role in the pathogenesis of catecholamine-induced insulin resistance *in vivo*.

Catecholamines induce insulin resistance in man (Rizza *et al.*, 1979, 1980; Deibert & Defronzo, 1980) and animals (Shikama & Ui, 1975; Sacca *et al.*, 1978). These studies *in vivo* suggest that this resistance arises from an interaction of catecholamines with insulin in insulin-sensitive tissues. A direct antagonistic action of catecholamines on insulin stimulation of glucose uptake has been demonstrated *in vitro* in skeletal muscle (Chiasson *et al.*, 1981) but in adipocytes an action on insulin-stimulated D-glucose transport could be seen only in the presence of high activities of adenosine deaminase (Green, 1983). It was not observed (Hagen & Ball, 1960; Cahill *et al.*, 1960; Flatt & Ball, 1964) when insulin and catecholamines were added simultaneously to fat-cells. Indeed there are reports that catecholamines even stimulate glucose

uptake beyond the level measured with insulin alone (Ludvigsen *et al.*, 1980; Häring *et al.*, 1981). In view of this contradiction between observations *in vivo* and *in vitro*, it seemed important to study the effect of isoprenaline and noradrenaline on insulin binding and insulin stimulation of 3-O-methylglucose transport in the adipocyte. Furthermore, since catecholamines are known to decrease cellular ATP levels *in vitro* (Hepp *et al.*, 1968; Bihler & Jeanrenaud, 1970; Angel *et al.*, 1971; Schwabe *et al.*, 1974) we measured ATP in cells pre-incubated with isoprenaline.

In the present paper we report a specific inhibition of insulin stimulation of D-glucose transport and a reduction in insulin binding at insulin concentrations smaller than 100 μ -units/ml after pre-incubation with catecholamines *in vitro*, which could be the mechanism underlying the observations *in vitro*. A preliminary report of these findings was given at the 1982 Meeting of the American

† To whom correspondence and reprint requests should be sent.

Diabetes Association (Häring *et al.*, 1982b) and the 1982 EASD meeting in Budapest (Kemmler *et al.*, 1982).

Methods

Fat-cells were isolated by the method of Rodbell (1964) from 180–220 g male Sprague–Dawley rats fed *ad libitum*. All incubations were performed in Krebs–Ringer–Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer [131 mM-NaCl/4.8 mM-KCl/2.5 mM-CaCl₂/1.2 mM-KH₂PO₄/1.2 mM-MgSO₄/25 mM-Hepes (pH 7.4)] containing 2.5% crystalline bovine serum albumin. Ascorbic acid (0.05 µg/ml) was added to this medium to prevent adrenochrome formation. DL-Isoprenaline hydrochloride and DL-noradrenaline hydrochloride were purchased from Serva, Heidelberg, Germany. Crystalline bovine insulin (Novo Industri, Bagsvaerd, Denmark) was used, 1 unit corresponding to 6.67 µmol.

3-O-Methylglucose transport

3-O-Methylglucose transport was measured by a modification of the method of Whitesell & Gliemann (1979) as described previously (Häring *et al.*, 1981a). Fat-cells were pre-incubated at 37°C in buffer in the presence or absence of catecholamines for 30 min and this was followed immediately by addition of insulin (5–1000 µ-units/ml) to this medium. After 20 min, 100 µl portions [(4.5–5.5) × 10⁶ cells/ml] were combined in a mixing pipette (Gilson Medical Electronics) with 200 µl of 3-O-methyl-D-glucose (final concentration 0.5 mM; 0.1 µCi of 3-O-methyl-D-[U-¹⁴C]glucose; Amersham International, Amersham, Bucks., U.K.). The transport experiment was terminated after 4 s by diluting the cells in 5 ml of cold NaCl solution (0.9 g/dl) containing phloretin (1 mM). Subsequently, cells and medium were separated by centrifugation through silicone oil at 1000 g for 30 s. The cell layer was removed from the oil with a pipette and the radioactivity associated with the cell pellet was determined. Apparent non-saturable 3-O-methylglucose influx, from physical diffusion and extracellular association of radioactivity, was estimated by measuring uptake during 4 s in the presence of 1 mM-phloretin. This kinetic component, which typically makes up 10% of the equilibrium value, was deducted from all uptake measurements. 3-O-Methylglucose accumulation measured during 4 s is expressed as a percentage of the 3-O-methylglucose accumulated at equilibrium in fat-cells after 20 min incubation at saturating insulin concentrations. From each incubation two samples were collected and all determinations were performed in duplicate. In cells that have maximum stimulation of transport activity the uptake

kinetics are not linear during the 4 s time interval used to estimate influx. Applying an extrapolation described by Kotyk & Janacek (1975), it could be shown that the actual initial uptake rate in cells that display maximum stimulation of transport activity is underestimated by approx. 15% (Häring *et al.*, 1981a). The 4 s time interval was nevertheless used because it only minimally deforms the insulin dose–response curve while allowing higher reproducibility of the measurement with the present technique than shorter time intervals.

Binding studies

Fat-cells [(4.5–5.5) × 10⁵/ml] were incubated with isoprenaline or noradrenaline in polyethylene vials at 37°C for 30 min. Subsequently, labelled insulin ([A14-¹²⁵I]insulin; sp. radioactivity 250 Ci/g; Novo Industri) in concentrations of 5 or 10 µ-units/ml alone or together with increasing concentrations of unlabelled insulin (5–1000 µ-units/ml) were added.

In preliminary experiments it had been established that insulin binding reached equilibrium after 4 min and remained stable for 30 min. Therefore, after a 20 min incubation, 400 µl portions were transferred into polyethylene centrifuge tubes and cells and medium were separated by centrifugation through dinonyl phthalate (Gliemann *et al.*, 1972). The tube was cut at the oil phase and the radioactivity associated with the cell phase was determined. From each incubation two samples were collected and all determinations were performed in duplicate. To determine non-specific binding, excess unlabelled insulin (1 unit/ml) was added together with the labelled insulin.

ATP determination

Portions of the cells used for 3-O-methylglucose transport determination or binding experiments were added to ice-cold trichloroacetic acid (final concentration 5 g/dl). Each sample was sonicated (Braun Sonic 125; 20 s, 100 W) and centrifuged at 40000 g and at 4°C for 10 min. After removal of trichloroacetic acid by diethyl ether extraction (3 × 7 vol.), the samples were concentrated by freeze-drying and ATP was determined by a luciferase assay (De Luca & McElroy, 1978).

Statistical significance was evaluated by using Student's *t* test for paired observations and two-tailed *P* values were calculated (**P* < 0.05; ***P* < 0.01). Values (*n*) given represent the number of independently performed experiments.

Results

Glucose transport and insulin binding after pre-incubation with isoprenaline

Incubation of cells with isoprenaline (10 µM) for 30 min before the addition of insulin caused a 30%

Fig. 1. Effects of isoprenaline on insulin stimulation of D-glucose transport and insulin sensitivity

(a) Effect of isoprenaline treatment on insulin stimulation of D-glucose transport. Adipocytes were pre-incubated for 30 min at 37°C in glucose-free medium in the absence (○ and ●) or the presence of isoprenaline (10 μ M; ▲). Subsequently, insulin was added for 20 min in the concentrations given on the abscissa. To a portion of the control cells, isoprenaline (10 μ M) was added simultaneously with insulin (●). D-Glucose uptake was measured with 3-O-methyl-D-[14 C]glucose as described in the Methods section and expressed as a percentage of equilibrium values obtained at saturating insulin concentrations after 20 min. The values represent the means of eight independently performed experiments $\pm$ S.E.M. At insulin concentrations above 10 μ -units/ml, the data obtained after pre-incubation with isoprenaline differed significantly from controls ($P < 0.01$). (b) Data obtained with cells pre-treated with isoprenaline (▲) as described above, or when isoprenaline was given simultaneously (●) with insulin, are expressed as percentages of the maximal insulin effect.

Fig. 2. Isoprenaline-induced inhibition of insulin binding in glucose-free medium

Adipocytes were pre-incubated in the absence (●) or the presence (▲) of isoprenaline (10 μ M) in glucose-free medium for 30 min at 37°C. Subsequently, 5 or 10 μ -units of [$A14^{125}I$]insulin/ml together with increasing amounts of unlabelled insulin as indicated on the abscissa were added and cells were incubated for 20 min at 37°C. Specific insulin binding was measured as described in the Methods section. The binding curve and the Scatchard plot of these data are shown. The data represent means of three independently performed experiments $\pm$ S.E.M. The specific binding of insulin differed significantly from the control at insulin concentrations of more than 5 μ -units/ml ($P < 0.05$).

reduction of the maximal response of the glucose transport system to insulin, whereas simultaneous addition of isoprenaline and insulin showed no

inhibitory effect (Fig. 1a). An ED_{50} (half-maximal stimulation of glucose transport by insulin) of in-

sulin in isoprenaline-treated cells of about $60 \mu\text{-units/ml}$, compared with $30 \mu\text{-units/ml}$ in control cells, was observed. When these values were expressed as percentages of the maximal insulin effect and were plotted as a function of the insulin concentration (Fig. 1*b*), there was still a rightward shift in the concentration-response curve to insulin. The data therefore indicated that pre-incubation of fat-cells with isoprenaline decreased both sensitivity and responsiveness of the glucose transport system to insulin. This could be due to an effect on insulin binding, since hormone-receptor interaction is the first step in signal transmission at the cellular level, and therefore insulin binding was investigated. As seen in Fig. 2(*a*), the isoprenaline ($10 \mu\text{M}$) given in the absence of glucose decreased insulin binding significantly at all concentrations of insulin ($5\text{--}1000 \mu\text{-units/ml}$). A Scatchard plot (Fig. 2*b*) of these data showed a parallel shift of the curve, which indicates an overall reduction of binding capacity. The typical curvilinear plot of insulin binding to adipocytes can be explained by either a model of negative co-operativity (DeMeys *et al.*, 1973, 1976) or a two-receptor model (Hammond *et al.*, 1972). Thus isoprenaline decreased either the number of the insulin receptors or the number of high- and low-affinity binding sites. Isoprenaline, however, is known to induce lipolysis in adipocytes and the non-esterified fatty acids that accumulate in the incubation medium could interfere with insulin binding. Therefore experiments were carried out where the cells were washed twice with 20 vol. of buffer, after the incubation period of 30 min with isoprenaline ($10 \mu\text{M}$), immediately before insulin was added. As shown in Fig. 3, an insulin resistance of D-glucose transport was then still observed, indicating that the catecholamine effect did not depend either on the presence of the catecholamine after induction of the effect or on the accumulation of a metabolite in the medium.

Insulin stimulation of D-glucose transport and insulin binding after pre-incubation with noradrenaline

The catecholamine-induced insulin resistance observed is in agreement with the one reported *in vivo*. Therefore we investigated whether the same effects were observed with the physiologically occurring catecholamine noradrenaline at physiological concentrations.

A decrease in sensitivity was observed at 1 nM -noradrenaline and at $0.1 \mu\text{M}$ a shift in the ED_{50} from 10 to $40 \mu\text{-units}$ of insulin/ml was observed together with a decrease in responsiveness of the glucose transport system to insulin by 20% (Fig. 4). The noradrenaline effects had an ED_{50} of 10 nM . When glucose was present in the medium at physiological concentrations (5 mM), or when in-

Fig. 3. *Independence of isoprenaline-induced inhibition of D-glucose transport from accumulated metabolites*

Isolated rat adipocytes were pre-incubated in the presence (▲ and △) or in the absence (●) of isoprenaline ($10 \mu\text{M}$) for 30 min at 37°C . Then control cells (●) and part of the cells pre-incubated with isoprenaline (△) were washed twice with 20 vol. of buffer. Subsequently, insulin was added in the concentrations given on the abscissa to the cells previously incubated with isoprenaline (▲ and △). To control cells, isoprenaline ($10 \mu\text{M}$) was added simultaneously with insulin (●). D-Glucose transport was measured as described in the Methods section after 20 min. Values are from one representative experiment, which was performed twice.

sulin was also added during the pre-incubation with noradrenaline ($0.1 \mu\text{M}$) in the physiological concentrations of 10 and $100 \mu\text{-units/ml}$ ($n = 4$; results not shown), a similar inhibition of glucose transport was still observed. Binding experiments with noradrenaline ($10 \mu\text{M}$) in the absence of glucose gave similar results to the ones obtained with isoprenaline as described above ($n = 3$; results not shown). Thus noradrenaline induced an insulin resistance *in vitro* at physiological glucose and insulin concentrations and this effect was concentration-dependent.

Insulin stimulation of D-glucose transport and insulin binding in the presence of different cellular ATP levels

The inhibition of insulin stimulation of D-glucose transport observed above might be explained by a catecholamine effect on cellular ATP levels since catecholamines reduce ATP levels in fat-cells *in vitro* (Hepp *et al.*, 1968; Bihler & Jeanrenaud, 1970; Angel *et al.*, 1971; Schwabe *et al.*, 1974), and a reduction in cellular [ATP] is known to decrease insulin binding (Häring *et al.*, 1981*a*) and inhibit

the insulin effect on glucose transport (Chandramouli *et al.*, 1977; Kono *et al.*, 1978; Siegel & Olefsky, 1980; Häring *et al.*, 1981a,b). We there-

Fig. 4. Dependence of the inhibition of insulin stimulation of D-glucose transport on the concentration of noradrenaline. Isolated rat adipocytes were pre-incubated for 30 min at 37°C in glucose-free buffer with 10 μM- (■, n = 1), 0.1 μM- (▲, n = 6), 10 nM- (Δ, n = 6) and 1 nM- noradrenaline (○, n = 2), or without noradrenaline (●). Then insulin in the concentrations indicated on the abscissa was added. To the control cells (●), insulin was added simultaneously with noradrenaline (10 μM). D-Glucose uptake was measured as described in the Methods section. Data are means of n independently performed experiments ± S.E.M. Inhibition due to noradrenaline in concentrations above 1 nM was statistically significant (*P* < 0.05) at insulin concentrations of more than 5 μ-units/ml from control values.

fore determined the ATP levels in our experiments and, as high glucose concentrations might prevent the [ATP] decrease caused by catecholamines (Angel *et al.*, 1971), we measured the ATP concentrations and the catecholamine effects on insulin-induced glucose transport at different glucose concentrations *in vitro*. Table 1 shows that addition of noradrenaline (0.1 μM) caused a marked decrease in ATP levels both in the absence of glucose (65%) and at 5 mM-glucose (45%) irrespective of insulin addition, whereas at 16 mM-glucose the ATP levels in the presence of insulin did not differ from those of control cells. Thus the inhibition of insulin stimulation of D-glucose transport by isoprenaline and noradrenaline in the absence of glucose or at low glucose concentrations could be due to the decrease in ATP levels observed above. Therefore we studied the effects of pre-incubation with noradrenaline at 16 mM-glucose, since any effects observed then should exclude changes induced by low ATP levels. As seen in Fig. 5, noradrenaline (0.1 μM) still reduced the sensitivity of D-glucose transport to insulin at 16 mM-glucose and the ED₅₀ was shifted from 45 μ-units/ml to 95 μ-units/ml. Only the decrease in responsiveness of D-glucose transport to insulin was not as marked at 16 mM-glucose as in the absence of glucose. The same effects were observed after pre-incubation with isoprenaline (10 μM) at 16 mM-glucose (n = 4; results not shown). It seems therefore that catecholamine-induced insulin resistance does not depend on a decrease in cellular ATP levels and that catecholamines interfere specifically with the insulin signal transmission to D-glucose transport in fat-cells.

Since insulin binding is also lowered by low ATP levels (Häring *et al.*, 1981a), the changes in insulin

Table 1. Noradrenaline-induced changes in ATP levels in the presence or in the absence of insulin at different concentrations of D-glucose

After isolated rat adipocytes were pre-incubated for 30 min at 37°C in medium containing 0, 5 or 16 mM-D-glucose in the absence or in the presence of noradrenaline (0.1 μM), samples were collected for determination of basal ATP. Insulin (1000 μ-units/ml) was subsequently added to part of the cells and new samples were taken after 20 min. ATP was measured by a luciferase method as described in the Methods section. Data express ATP levels (nmol/10⁵ cells) and are means of seven independently performed experiments ± S.E.M. The ATP levels of noradrenaline pretreated cells in 0 and 5 mM-D-glucose-containing buffer were significantly different from levels in their respective controls (*P* < 0.05).

Glucose (mM)	ATP (nmol/10 ⁵ cells)					
	0		5		16	
Insulin (1000 μ-units/ml)	-	+	-	+	-	+
Without noradrenaline	0.66 ± 0.26	0.86 ± 0.20	0.67 ± 0.23	0.73 ± 0.14	0.86 ± 0.21	0.79 ± 0.17
With noradrenaline (0.1 μM)	0.24 ± 0.09	0.30 ± 0.14	0.41 ± 0.13	0.41 ± 0.06	0.64 ± 0.23	0.77 ± 0.28

Fig. 5. Noradrenaline-induced inhibition of insulin stimulation of D-glucose transport at normal ATP levels. Isolated rat adipocytes were pre-incubated for 30 min at 37°C in medium containing 5 (●) or 16 (▲) mM-D-glucose in the absence (control) or in the presence of noradrenaline (10 μM) (○, 5 mM-glucose; △, 16 mM-glucose). Then insulin in the concentrations given on the abscissa was added and D-glucose transport was measured as described in the Methods section. Data are means of four independently performed experiments $\pm$ S.E.M. Significant differences (* P < 0.05) from respective controls are indicated.

binding (Fig. 2) could equally have been due to a catecholamine-induced [ATP] decrease. Pre-incubation with noradrenaline (10 μM) at 16 mM-glucose, however, also caused a decrease in insulin binding (Fig. 6a), although in contrast with the experiments without glucose (Fig. 2), a decrease in binding by 35% was seen at 16 mM-glucose only at insulin concentrations of less than 100 μ-units/ml.

The Scatchard plot (Fig. 6b) suggested that at these low concentrations there was mainly a decrease in binding affinity but not in the capacity, as both binding curves converged to a common line. If the model of negative co-operativity is used, a decrease in high-affinity binding has to be assumed (DeMeyts *et al.*, 1973, 1976). If the two-receptor model is accepted (Hammond *et al.*, 1972) then this indicates a decrease in high-affinity binding sites. Preincubation with isoprenaline (0.1 μM) also caused inhibition of high-affinity binding by 35% ($n = 4$; results not shown). Thus a decrease in insulin binding in high-affinity sites might cause the catecholamine-induced insulin resistance observed above.

Time course of the catecholamine effect

As seen in Fig. 6(a), a decrease in insulin binding was also observed with simultaneous addition of noradrenaline and insulin and this is not consistent with the slight increase in glucose transport that

Fig. 6. Noradrenaline-induced inhibition of insulin binding at normal ATP levels.

Adipocytes were pre-incubated in the absence (● and △) or in the presence (▲) of noradrenaline (10 μM) in 16 mM-D-glucose-containing medium for 30 min at 37°C. Subsequently, 5 or 10 μ-units of [$A14^{125}I$]insulin/ml and increasing amounts of unlabelled insulin as indicated on the abscissa were incubated with the cells for 20 min at 37°C. To a portion of the control cells 10 μM-noradrenaline was added simultaneously with insulin (△). Specific insulin binding was measured as described in the Methods section. The binding curve (a) and the Scatchard plot of these data (b) are shown. Data are the means of five independently performed experiments $\pm$ S.E.M. The S.E.M. values of the data where noradrenaline was added simultaneously with insulin (△) were omitted for clarity. The specific binding of insulin in cells pre-incubated with noradrenaline differed significantly from that of controls at insulin concentrations of less than 100 μ-units/ml (P < 0.05).

Fig. 7. Time course of the noradrenaline effects on D-glucose transport stimulation by insulin

Isolated rat adipocytes were pre-incubated for the time indicated on the abscissa in buffer containing 5 mM-glucose at 37°C in the absence (control) or in the presence of noradrenaline (0.1 μM). Subsequently, 5 μ-units (▲), 25 μ-units (○), 100 μ-units (●) or 300 μ-units (Δ) of insulin were added and D-glucose uptake was measured after 20 min as described in the Methods section. Data are means of four independently performed experiments ± S.E.M. The S.E.M. values of points between 0 and 30 min were omitted for clarity. The significance of the difference seen in the data obtained with cells after pre-incubation with noradrenaline as compared with their respective controls is indicated (* $P < 0.05$; ** $P < 0.01$).

was observed when isoprenaline and insulin were added simultaneously (Fig. 1a). We therefore investigated the time course of the inhibitory catecholamine effect by giving noradrenaline (0.1 μM) either simultaneously with insulin or up to 30 min before insulin. As shown in Fig. 7, simultaneous addition of noradrenaline and insulin enhanced the insulin-stimulated glucose transport compared with insulin without catecholamine, but only at concentrations of at least 100 μ-units of insulin/ml. No stimulatory noradrenaline effect was seen at insulin concentrations between 5 and 25 μ-units/ml. These results agree with our data from the insulin-binding experiments (Fig. 6) since no significant inhibition of insulin binding was observed at insulin concentrations of 100 μ-units/ml or more. After 10 min of pre-incubation, however, there was an inhibitory effect of noradrenaline on glucose transport, which was again highest at high insulin concentrations. An insulin resistance of D-glucose transport was therefore induced by pre-

incubation of the fat-cells with catecholamines for more than 10 min.

Discussion

The mechanism by which counter-regulatory hormones interfere with insulin on target cells was up to now believed to be mainly an interaction with synthesis and regulation of the enzymes of intermediary metabolism (Porte & Halter, 1981). Our study, however, suggests that they might already act upon the first step of insulin signal transmission, since an inhibitory effect of catecholamines on insulin binding to the receptor could be demonstrated. Pre-incubation with catecholamines, moreover, inhibited D-glucose transport. These effects differed according to the time of administration of the catecholamine to the cells. The catecholamine effect also depended on the glucose concentration that was used, as this determined the ATP levels that were maintained after addition of catecholamines.

It is known that catecholamines can cause a decrease in intracellular ATP levels via induction of lipolysis (Hepp *et al.*, 1968; Bihler & Jeanrenaud, 1970; Angel *et al.*, 1971; Schwabe *et al.*, 1974). Furthermore a lack of ATP interferes with all energy-dependent steps of insulin binding such as the rapid decrease in insulin dissociation after insulin binding (Häring *et al.*, 1980, 1981a). Furthermore low ATP levels could interfere with the phosphorylation reactions and the kinase activity of the insulin receptor (Avruch *et al.*, 1976; Seals *et al.*, 1979; Häring *et al.*, 1982a). We confirmed this decrease in ATP levels due to catecholamines at low glucose concentrations (0–5 mM-D-glucose; Table 1), so that the effects observed with catecholamines under these conditions could be mainly due to the decrease in ATP levels. It was therefore necessary to measure the effects due to catecholamines without a decrease in intracellular ATP levels in our experiments. We solved this problem by studying the effects of catecholamines in the presence of high glucose concentrations, as there was no decrease in ATP levels at 16 mM-glucose in the presence of insulin. In the absence of insulin, the ATP levels were reduced by 24% at 16 mM-glucose but no change of binding is observed at this small decrease of ATP (Häring *et al.*, 1981b). At 16 mM-glucose catecholamines still caused a significant decrease in sensitivity and responsiveness of the glucose transport system to insulin (Fig. 5), which was of the same order of magnitude as the effects seen without glucose (Figs. 1, 3 and 4). The catecholamine effect on binding at high glucose concentrations was an inhibition of high-affinity binding (Fig. 6), whereas in the presence of low ATP levels there was a parallel shift in the

Scatchard plot (Fig. 2), which suggests an overall decrease in binding sites. Even though the catecholamine effect at normal ATP levels was smaller, it was statistically significant and affected binding at physiological insulin concentrations. The mechanism of this catecholamine action on the insulin receptor, however, remains to be investigated, although we have evidence that the catecholamine effect is mediated by an increase in cyclic AMP levels (Kirsch *et al.*, 1983). A cyclic AMP-dependent kinase reaction could modulate the insulin-induced protein phosphorylation or the phosphorylation of the insulin receptor in the intact fat-cell (Avruch *et al.*, 1976; Benjamin & Clayton, 1978; Seals *et al.*, 1979; Häring *et al.*, 1982a), even though no influence of cyclic AMP on phosphorylation of a partially purified receptor has so far been observed (Häring *et al.*, 1982a).

Furthermore one should consider to what extent the catecholamine effect on binding caused the changes in D-glucose transport. According to the 'spare receptor concept' (Kahn, 1978), the decrease in the affinity of binding agrees well with the loss of sensitivity of D-glucose transport that was observed at insulin concentrations between 10 and 300 μ -units/ml (Fig. 5).

However, whereas a decrease in binding affinity would explain most of the effects observed with pre-incubation with catecholamines, it could not explain all the changes seen. The decrease in responsiveness at high insulin concentrations (more than 300 μ -units/ml; Fig. 5) cannot be caused by a mere decrease in affinity of binding (Kahn, 1978), so that a post-insulin-receptor mechanism is probably also involved in the inhibitory catecholamine effects on D-glucose transport.

Experiments where catecholamines were added simultaneously with insulin also give evidence of a post-insulin-receptor effect of catecholamines but one that is of a stimulatory kind. There, we found in agreement with Ludvigsen *et al.* (1980) that catecholamines led to an increase in glucose transport at insulin concentrations above 100 μ -units/ml (Fig. 7). Under these conditions, however, catecholamine administration caused no increase in insulin binding (Fig. 6), so that this stimulatory effect of catecholamines on D-glucose transport is not mediated by the insulin receptor. The fact that catecholamines, when given alone, increase the basal glucose transport, as described by Ludvigsen *et al.* (1980) and confirmed in our laboratory (D. Kirsch, W. Kemmler & H. U. Häring, unpublished work), lends further support to this hypothesis of a post-insulin-receptor action of catecholamines in fat-cells *in vitro*. The main feature of the catecholamine effect on glucose transport *in vivo*, however, is an insulin resistance, and therefore the stimulatory post-insulin-receptor effects of

catecholamines on D-glucose transport may be a phenomenon only *in vitro*.

In summary, our results indicate that catecholamines, including the physiologically occurring noradrenaline, can, when given in a physiological concentration range at normal insulin and ATP concentrations, induce insulin resistance of the glucose transport system in fat-cells *in vitro*. Binding studies furthermore show that the catecholamine effects on D-glucose transport under those conditions are mostly due to a reduction of high-affinity binding. This may therefore be the mechanism for the antagonism between catecholamines and insulin observed in peripheral tissues *in vivo* (Shikama & Ui, 1975; Sacca *et al.*, 1978; Rizza *et al.*, 1979, 1980; Deibert & DeFronzo, 1980).

The catecholamine inhibition of insulin binding can be considered as an example of receptor regulation by hormones. A well-known phenomenon is 'receptor autoregulation' in which high hormone concentrations lead to a 'down-regulation' of the receptor, which can be observed with, e.g., insulin (Gavin *et al.*, 1974). A heterologous receptor regulation by hormones has been described for follitropin, which increases androgen receptors in Sertoli cells (Fritz & Rommerts, 1976), and for somatotropin and glucocorticoids, which have a negative effect on insulin receptors *in vivo* (Kahn *et al.*, 1978).

Insulin itself down-regulates insulin-like growth factor I receptors (Rosenfeld & Dollar, 1982) and thyroxine increases catecholamine receptors (Landsberg, 1977). These are examples where peptide hormones regulate receptors of peptide or steroid hormones or vice versa. Therefore, the different classes of hormones with differently localized binding sites and second-messenger mechanisms seem to employ the same principle of receptor regulation. The regulation of insulin receptors by catecholamines provides further evidence that this is a general principle of endocrinological regulation.

We thank Dr. C. R. Kahn and Dr. M. F. White for help during the preparation of this manuscript, Ms. Eva Rattenhuber and Ms. Joanne Mushack for expert technical assistance and Ms. Sue Tracey, Ms. Barbara Cornell and Ms. Dagmer Lehmeier for secretarial assistance.

References

- Angel, A., Desai, K. & Halperin, M. L. (1971) *Metab. Clin. Exp.* **20**, 87-98
- Avruch, J., Leone, G. R. & Martin, D. B. (1976) *J. Biol. Chem.* **251**, 1511-1515
- Benjamin, G. J. & Clayton, N. C. (1978) *J. Biol. Chem.* **253**, 1700-1709

- Bihler, C. & Jeanrenaud, B. (1970) *Biochim. Biophys. Acta* **202**, 496–506
- Cahill, G. F., Jr., Leboeuf, B. & Flinn, R. B. (1960) *J. Biol. Chem.* **235**, 1246–1250
- Chandramouli, V., Milligan, M. & Carter, R. H. (1977) *Biochemistry* **16**, 1151–1158
- Chiasson, J. L., Shikama, H., Chu, D. T. W. & Exton, J. H. (1981) *J. Clin. Invest.* **68**, 706–713
- Deibert, C. & DeFronzo, R. A. (1980) *J. Clin. Invest.* **65**, 717–721
- De Luca, M. & McElroy, W. D. (1978) *Methods Enzymol.* **57**, 3
- DeMeyts, P., Roth, I., Neville, D. M., Jr., Gavin, J. R., III & Lesniak, M. A. (1973) *Biochem. Biophys. Res. Commun.* **55**, 154–161
- DeMeyts, P., Bianco, A. R. & Roth, J. (1976) *J. Biol. Chem.* **251**, 1877–1888
- Flatt, J. P. & Ball, E. G. (1964) *J. Biol. Chem.* **239**, 675–685
- Fritz, I. B. & Rommerts, F. G. (1976) *J. Reprod. Fertil.* **46**, 17–23
- Gavin, J. R., III, Roth, J., Neville, D. M., Jr., DeMeyts, P. & Buell, D. N. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84–88
- Gliemann, J., Osterlind, K., Vinten, J. & Gammeltoft, S. (1972) *Biochim. Biophys. Acta* **286**, 1–9
- Green, A. (1983) *FEBS Lett.* **152**, 261–264
- Hagen, J. H. & Ball, E. G. (1960) *J. Biol. Chem.* **235**, 1545–1549
- Hammond, J. M., Jarett, L., Marit, I. K. & Doughaday, W. H. (1972) *Biochem. Biophys. Res. Commun.* **49**, 1122–1128
- Häring, H. U., Brocks, D. C., Siess, E. A. & Kemmler, W. (1980) in *Insulin, Chemistry, Structure and Function of Insulin and Related Hormones* (Brandenburg, D. & Wollmer, A., eds.), pp. 469–474, de Gruyter Co., Berlin and New York
- Häring, H. U., Biermann, E. & Kemmler, W. (1981a) *Am. J. Physiol.* **240**, E556–E565
- Häring, H. U., Rinninger, F. & Kemmler, W. (1981b) *FEBS Lett.* **132**, 235–238
- Häring, H. U., Kasuga, M. & Kahn, C. R. (1982a) *Biochem. Biophys. Res. Commun.* **108**, 1538–1545
- Häring, H. U., Baumgarten, M., Deufel, T. & Kemmler, W. (1982b) *Diabetes* **31**, 125A
- Hepp, D., Challoner, D. R. & Williams, R. H. (1968) *J. Biol. Chem.* **243**, 2321–2327
- Kahn, C. R. (1978) *Metab. Clin. Exp.* **27**, 1893–1902
- Kahn, C. R., Goldfine, I. D., Neville, D. M., Jr. & DeMeyts, P. (1978) *Endocrinology* **103**, 1054–1066
- Kemmler, W., Häring, H. U., Baumgarten, M. & Kirsch, D. (1982) *Diabetologia* **23**, 179
- Kirsch, D., Häring, H. U. & Kemmler, W. (1983) *Biochem. Biophys. Res. Commun.* **115**, 378–405
- Kono, T., Robinson, F. W., Sarver, J. A., Vega, F. V. & Pointer, R. H. (1978) *J. Biol. Chem.* **252**, 2226–2233
- Kotyk, A. & Janacek, K. (1975) in *Cell Membrane Transport: Principles and Techniques*, p. 263, Plenum Press, New York
- Landsberg, L. (1976) in *The Year in Endocrinology* (Ingbar, S. H., ed.), Plenum Press, New York
- Ludvigsen, C., Jarett, L. & McDonald, J. M. (1980) *Endocrinology* **106**, 786–790
- Porte, D., Jr. & Halter, J. B. (1981) in *Textbook of Endocrinology*, 6th edn., (Williams, R. H., ed.), Saunders Co., Philadelphia
- Rizza, R., Haymond, M., Cryer, P. E. & Gerich, J. (1979) *Am. J. Physiol.* **237**, E356–E362
- Rizza, R. A., Cryer, P. E., Haymond, M. W. & Gerich, J. E. (1980) *J. Clin. Invest.* **65**, 682–689
- Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
- Rosenfeld, R. G. & Dollar, L. A. (1982) *J. Clin. Endocrinol. Metab.* **55**, 434–440
- Sacca, L., Sherwin, R. & Felig, P. (1978) *Am. J. Physiol.* **235**, E287–E290
- Schwabe, U., Schonhofer, P. S. & Ebert, R. (1974) *Eur. J. Biochem.* **46**, 537–545
- Seals, J. R., McDonald, J. M. & Jarett, L. (1979) *J. Biol. Chem.* **254**, 6991–6996
- Shikama, H. & Ui, M. (1975) *Am. J. Physiol.* **229**, 955–961
- Siegel, J. & Olefsky, J. M. (1980) *Biochemistry* **19**, 2183–2190
- Whitesell, R. R. & Gliemann, J. (1979) *J. Biol. Chem.* **254**, 5276–5283