The fate of insulin in cardiac muscle

Studies on isolated muscle cells from adult rat heart

Jürgen ECKEL and Hans REINAUER

Biochemical Department, Diabetes Research Institute, 4000 Düsseldorf 1, Federal Republic of Germany

(Received 21 May 1982/Accepted 8 June 1982)

Isolated muscle cells from adult rat heart were used to study myocardial degradation of insulin and the reactions after the initial binding event. After 60 min of association at 37°C, 90% of specifically bound insulin could be dissociated from the cells; this fraction remained unaltered under steady-state conditions (up to 180 min). To assess the nature of cell-associated radioactivity, cardiocytes were solubilized and filtered on Sephadex G-50. After 5 min of association only intact insulin was observed, whereas under steady-state conditions 4% of ^{125}I -labelled insulin bound to the cells was degraded to iodotyrosine-containing fragments. The K_m for insulin degradation by isolated heart cells was estimated to be $1.75 \times 10^{-7} \text{ M}$. Receptor-mediated insulin degradation was studied by examination of the nature of radioactivity released by the cells after different times of association. After 5 min 83% of dissociating material consisted of intact insulin, whereas this fraction decreased to 50% under steady-state conditions. Treatment of cells with the lysosomotropic agent chloroquine (0.1 mM) significantly decreased the fraction that was eluted at the internal column volume. This study demonstrates that insulin degradation by the heart cell occurs by a receptor-independent and a receptor-dependent mechanism. The latter may involve internalization and a lysosomal pathway.

Evidence indicates that the interaction of insulin with target cells involves a number of different processes and cannot be described as a bimolecular reversible reaction (Kahn & Baird, 1978; Gorden *et al.*, 1980). Initially, the hormone interacts with a specific receptor site on the plasma membrane to form a hormone–receptor complex (Kahn, 1976). After this, these complexes may be translocated within the plasma membrane (Carpentier *et al.*, 1978; Kono *et al.*, 1977) and/or internalized by adsorptive pinocytosis as suggested by Carpentier *et al.* (1979a).

The functional role of the internalized insulin is at present uncertain. Intracellular receptors have been reported on Golgi structures (Bergeron *et al.*, 1978), nuclei (Goldfine & Smith, 1976) and endoplasmic reticulum (Kahn, 1976). Other investigators observed a preferential localization of internalized insulin in lysosomes (Carpentier *et al.*, 1979a,b). The latter process would mediate insulin degradation and link

it to receptor binding, as shown by Terris & Steiner (1975). More recently, receptor-mediated degradation has been demonstrated in adipocytes (Gliemann & Sonne, 1978; Olefsky *et al.*, 1979), possibly involving a lysosomal pathway (Marshall & Olefsky, 1979). The formation of insulin fragments inside a target cell has raised the question of biological activity of these fragments (Goldfine, 1977) and led to the suggestion of a possible relationship between degradation and hormone action.

Insulin degradation has been studied in many different tissues, including hepatocytes (Terris & Steiner, 1975; Le Cam *et al.*, 1975), adipocytes (Hammond & Jarret, 1975; Kahn & Baird, 1978; Gliemann & Sonne, 1978; Goldstein & Livingston, 1980), cultured human lymphocytes (Sonne & Gliemann, 1980), mononuclear cells (Powers *et al.*, 1980), kidney (Duckworth, 1978) and skeletal muscle plasma membranes (Yokono *et al.*, 1979). However, essentially no information is at present available on the fate of insulin in cardiac muscle, though it is known to be one of the major targets for insulin action (Morgan *et al.*, 1959; Chain *et al.*, 1969; Fischer, 1971; Kones & Phillips, 1975; Cheung *et al.*, 1978).

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; V_0 , V_{ins} , V_A and V_i , the gel-filtration elution volumes for Blue Dextran, insulin, insulin A chain and the column internal volume, respectively.

The aim of the present work was to study the events that follow initial formation of insulin-receptor complexes and to gain some insights into myocardial degradation of insulin. Isolated muscle cells from adult rat heart, which retain a number of metabolic functions and possess specific receptors for insulin (Eckel & Reinauer, 1980*a,b*, 1981; Eckel *et al.*, 1982), have been used to approach these problems. The results suggest that insulin degradation in the heart muscle is mediated by both receptor-independent and receptor-dependent mechanisms. The latter process may involve internalization and degradation through a lysosomal pathway.

Materials and methods

Chemicals

Pig [^{125}I]iodotyrosine-A14]insulin (sp. radioactivity 200–300 $\mu\text{Ci}/\mu\text{g}$) and pig monocomponent insulin were obtained from Novo, Bagsvaerd, Denmark. Collagenase (EC 3.4.24.3) was supplied by Boehringer, Mannheim, Germany. Bovine serum albumin (fraction V) and Hepes were purchased from Serva, Heidelberg, Germany. Hyaluronidase (EC 3.2.1.35), urea, digitonin, Triton X-100 and chloroquine were obtained from Sigma, München, Germany. Sephadex G-50 and Blue Dextran were from Pharmacia, Freiburg, Germany. Silicone oil AR 200 (density 1.04 g/ml) was purchased from Wacker Chemie, München, Germany. All other chemicals were analytical grade and obtained from Merck, Darmstadt, Germany.

Isolation of heart cells

Male Wistar rats (280–320 g) were used in all experiments. All studies were performed in the morning on animals that had free access to food and drinking water. Myocytes were isolated by perfusion of the heart with collagenase as detailed previously (Eckel & Reinauer, 1980*a*). The isolated cells were washed three to five times with Hepes buffer (composition: NaCl 130 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, Hepes 25 mM, glucose 5 mM, bovine serum albumin 20 g/l, pH 7.6, equilibrated with O_2). The concentration of free Ca^{2+} in this buffer was found to be 0.16 mM as measured with an ion-selective electrode (Orion Research, Cambridge, MA, U.S.A.). The cell suspension was stored under O_2 at room temperature until use. Cell numbers were determined in a Fuchs–Rosenthal chamber; cell viability as judged by determination of the percentage of rod-shaped cells was 80–90%.

Insulin binding assay

Isolated cardiac myocytes (6×10^4 – 8×10^4 cells/ml) were suspended in Hepes buffer, pH 7.6, and incubated with ^{125}I -labelled insulin at 37°C in a final

volume of 0.5 ml. Incubations were terminated by the oil centrifugation technique as detailed elsewhere (Eckel & Reinauer, 1980*a*). All binding experiments were performed in triplicate; duplicate tubes were run in parallel to determine non-specific binding, which was considered as the amount of ^{125}I -labelled insulin bound in the presence of unlabelled insulin (1 μM). All binding data reported here have been corrected for non-specific binding.

Trichloroacetic acid-solubility assay for insulin degradation

Degradation of ^{125}I -labelled insulin in the incubation medium was measured by an increase in trichloroacetic acid solubility of the tracer. The assay consisted of incubating cardiocytes with the labelled hormone and separating the cells from the incubation mixture by centrifugation at 20 g for 5 min. Samples of the supernatant were added to Tris/HCl buffer (50 mM, containing bovine serum albumin, 5 g/l), pH 7.0, and after addition of trichloroacetic acid (final concn. 5%, w/v) precipitation was allowed to occur for 10 min at 4°C. After centrifugation (1500 g for 5 min), both supernatant and pellet were counted for radioactivity in a gamma spectrometer. The percentage degraded was calculated relative to control samples incubated in the absence of cells. In some experiments the degrading activity of the incubation mixture after removal of the cells was determined in parallel incubations and subtracted from total degradation when indicated.

Gel filtration

Gel filtration was performed by applying 1 ml samples to a Sephadex G-50 column (1.6 cm $\times$ 70 cm). The column was eluted with acetic acid (1 M)/urea (4 M)/0.1% Triton X-100 at a flow rate of 13.8 ml/h. Fractions (1.9 ml) were collected and the radioactivity was determined. Recovery of total radioactivity applied exceeded 95% in every case. Representative gel profiles are shown; however, each experiment has been repeated three to five times to ensure consistency of results. The column was calibrated with Blue Dextran (V_0), ^{125}I -labelled insulin (V_{ins}), ^{125}I -labelled A chain (V_{A}) and Na^{125}I (V_{I}).

To determine the nature of cell-associated radioactive material, heart cells were washed three times with Hepes buffer at 4°C and then solubilized in 1 ml of a solution containing urea (6 M), acetic acid (1 M) and 0.2% Triton X-100. After centrifugation (2 min at 10000 g), 1 ml of the supernatant was analysed by column chromatography.

Results

Insulin binding at steady state

As previously described, insulin specifically binds

to isolated cardiocytes in a time- and temperature-dependent reaction (Eckel & Reinauer, 1980a). As shown in Fig. 1, at 37°C maximum binding was reached by 60 min and remained constant up to at least 180 min. It should be noted that the initial cell viability (80–90%) decreases by 10–20% with longer incubation periods; no difference, however, has been observed between insulin-treated and untreated cells. Thus, under appropriate incubation conditions a constant concentration of receptor-bound insulin can be maintained for at least 2 h. Addition of excess unlabelled insulin (1 μ M) induced a rapid dissociation of bound 125 I-labelled insulin from the myocytes (Fig. 1). After 10 min of association 97% of specifically bound hormone could be dissociated from the cells; this fraction decreased to 90% after 60 min of association, but remained unaltered under steady-state conditions. These data suggest that there is no tendency for irreversible binding and that all of the receptor-bound hormone remains fully accessible from outside the cell even at physiological temperatures.

Insulin bound to a target cell may become processed to higher- and lower-molecular-weight fragments, as suggested by Kahn & Baird (1978). In order to study the chemical nature of the cell-

Fig. 1. Binding of 125 I-labelled insulin to isolated heart cells as a function of time and dissociation of bound insulin by addition of excess insulin

Heart cells (0.8×10^5 cells/ml) were incubated with 125 I-labelled insulin (49 pM) in Hepes buffer. At the indicated times (●) an excess of unlabelled insulin (1 μ M) was added and incubation continued for 60 min (○) and 120 min (△) at 37°C. Specific binding was determined as detailed in the Materials and methods section. Specific binding at the beginning of dissociation was measured after addition of an equal volume of buffer (●). The data reported are mean values $\pm$ S.E.M. for three separate experiments.

associated radioactivity, isolated heart cells were allowed to associate with 125 I-labelled insulin for different lengths of time, solubilized and subjected to gel filtration on Sephadex G-50. After 5 min of association the elution profile revealed only one peak

Fig. 2. Gel-filtration profiles of radioactivity extracted from isolated cardiac myocytes

Cardiocytes (1×10^5 cells/ml) were incubated with 125 I-labelled insulin (90 pM) in a total volume of 8 ml. At the indicated times the cells were separated from the incubation medium, washed, extracted and chromatographed as described in the text. The elution profile of non-specific binding is shown in panel (b) (○).

co-migrating with ^{125}I -labelled insulin standard (Fig. 2a), whereas after 60 min about 4% of bound radioactivity was eluted just beyond the ^{125}I peak, representing low-molecular-weight material, most probably iodotyrosine-containing fragments (Freychet *et al.*, 1972). This fraction, however, was not significantly altered under steady-state conditions (up to 180 min) (Fig. 2c). An increasing amount of radioactivity also appeared in the void volume of the column, reaching about 36% of total after 180 min of incubation. Kahn & Baird (1978) suggested that this high-molecular-weight material may result from a physiologically relevant interaction of insulin with its receptors on the surface of target cells. However, incubation of ^{125}I -labelled insulin in Hepes buffer in the absence of cells resulted in the formation of high-molecular-weight material, suggesting that this process is unrelated to insulin metabolism (results not shown). Moreover, V_0 material represents more than 75% of non-specifically bound insulin after 60 min of association (Fig. 2b) and the increase in the V_0 peak is paralleled by an increase in non-specific binding (not shown in Fig. 2). From our data it can be calculated that essentially all the high-molecular-weight material extracted from the cells after 60 min is due to non-specific binding and that more than 96% of intact insulin is specifically bound to the cardiocytes under these conditions.

Insulin degradation in the medium

The total degrading activity of isolated cardiac myocytes was determined from the production of trichloroacetic acid-soluble radioactivity in the medium after incubation of cells with ^{125}I -labelled insulin. Insulin degradation was linearly related to the cell number up to 2.4×10^5 cells/ml and increased in a linear fashion up to 30 min of incubation (results not shown). In contrast, linearity of insulin binding was only observed up to 1.2×10^5 cells/ml, as previously described (Eckel & Reinauer, 1980a).

Some degrading activity was also detected in the incubation medium after removal of the cells. This activity could not be inhibited by high concentrations of insulin ($1 \mu\text{M}$) and probably results from a leakage of intracellular degrading activity owing to an increase in the number of round cells during incubations at 37°C . Consistently, a suspension of 100% round cells, prepared by addition of digitonin, exhibits about 8 times as much degrading activity as do rod-shaped cells (results not shown).

In order to determine the affinity of the insulin-degrading system(s), isolated heart cells were incubated with insulin concentrations ranging from 100 pM to $0.2 \mu\text{M}$. The K_m for insulin degradation was obtained from a Hanes plot of the data (Fig. 3) and estimated to be $1.75 \times 10^{-7} \text{ M}$. This value is

Fig. 3. Degradation of insulin as a function of substrate concentration

Heart cells (1.2×10^5 – 1.6×10^5 cells/ml) were incubated with ^{125}I -labelled insulin (100 pM) and increasing concentrations of unlabelled insulin (up to $0.2 \mu\text{M}$) for 30 min at 37°C . The rate of insulin degradation, v , was measured by an increase in trichloroacetic acid solubility of the tracer as outlined in the text. In parallel incubations the degrading activity of the incubation medium after removal of the cells was determined and subtracted from total degradation. Data are presented as a Hanes plot. K_m was calculated from a regression line.

identical with that reported by Yokono *et al.* (1979) for insulin-degrading activity of plasma membranes of rat skeletal muscle, and very similar to that of the isolated hepatocyte system (Le Cam *et al.*, 1975). The data in Fig. 3 have been corrected for the degrading activity found in the incubation medium after removal of the cells; thus the reported degradation only results from an interaction of insulin with the isolated heart cell.

Degradation of receptor-bound insulin

In order to rule out a possible involvement of receptor binding in insulin degradation by isolated cardiac myocytes, we have studied the nature of radioactivity released by the cells after different times of association. After 5 min 83% of the material that dissociated from the cells co-eluted with iodoinsulin, whereas 9% co-eluted with ^{125}I , on gel filtration (Fig. 4a). Under steady-state conditions

Fig. 4. Gel-filtration profiles of radioactivity released from isolated heart cells

Cells were incubated with ^{125}I -labelled insulin (90 pM) for the indicated times, centrifuged and washed three times with Hepes buffer at 4°C . After resuspension in 1.6 ml of fresh buffer, dissociation was allowed to take place for 20 min at 37°C . The medium was recovered and applied to the Sephadex G-50 column. Elution was performed as outlined in the text.

(60 min) four peaks of radioactivity could be detected (Fig. 4b): 50% co-migrated with ^{125}I -labelled insulin standard, 8% was found in the V_0 region, and 17 and 25% eluted in the position of the A-chain and at the internal column volume respectively. These data suggest that insulin bound to the receptor is an efficient substrate for insulin degradation by the isolated heart cell; insulin bound at steady state, however, seems to be more readily available for degradation than is the initially associated hormone.

Effect of chloroquine on insulin binding and degradation

Evidence suggests that receptor-mediated degradation of insulin involves internalization of the hormone and a lysosomal pathway (Marshall & Olefsky, 1979; Suzuki & Kono, 1979). Alternatively, the receptor may transfer insulin molecules to

a degrading site in the plasma membrane, or the receptor and the degrading enzyme represent subunits of the same macromolecule (Gliemann & Sonne, 1978).

In an attempt to evaluate these different mechanisms, we have studied the receptor-mediated degradation of insulin after treatment of cells with chloroquine, which is a well-documented lysosomotropic inhibitor of proteolysis (De Duve *et al.*, 1974; Wibo & Poole, 1974). The gel-filtration pattern of radioactivity dissociated from control and chloroquine-treated cells after association with ^{125}I -labelled insulin for 60 min is presented in Fig. 5. The fraction eluted at the internal column volume, V_i , significantly decreased from 32.2% to 11.0% of total radioactivity after treatment of cells with chloroquine. It should be noted that no difference in viability of treated and untreated cells has been observed under our experimental conditions. In view of the specific inhibition of lysosomal degradative function by chloroquine (Marshall & Olefsky, 1979), our data strongly suggest the involvement of lysosomes in insulin degradation by isolated cardiac myocytes.

Additional evidence for this assumption is presented in Fig. 6. Incubation of isolated heart cells with ^{125}I -labelled insulin in the presence of chloroquine results in a progressive increase of cell-associated radioactivity, reaching 193% of the control value

Fig. 5. Gel-filtration profile of radioactivity released from isolated heart cells after chloroquine treatment

Cells were incubated for 30 min in the presence (○) and absence (●) of chloroquine (0.1 mM). ^{125}I -labelled insulin (62 pM) was then allowed to associate for 60 min. Separation of free hormone, dissociation of bound radioactivity and gel filtration were performed as described in the legend to Fig. 4.

Fig. 6. Effect of chloroquine on the time course of insulin binding to isolated heart cells

Cardiocytes (0.6×10^5 cells/ml) were preincubated for 30 min in the presence (○) or absence (●) of chloroquine (0.1 mM). After preincubation, samples of the cell suspension were further incubated for the indicated time intervals in the presence of ^{125}I -labelled insulin (25 pM) and specific binding was determined as detailed in the text. The results represent means $\pm$ S.E.M. for three separate experiments.

Fig. 7. Effect of chloroquine on the dissociation of cell-associated insulin

Isolated heart cells were incubated for 120 min with ^{125}I -labelled insulin (41 pM) after preincubation for 30 min in the presence (○) or absence (●) of chloroquine (0.1 mM). Unlabelled insulin (1 μM) was then added, and the dissociation of radioactivity was monitored by removing samples from the incubation mixture at the indicated times and determination of specific binding as outlined in the text. All data points are mean values of three separate experiments.

after 3 h of incubation. A similar observation has been reported by Marshall & Olefsky (1979) for isolated adipocytes and by Tsai & Seeman (1981) for cultured hepatoma cells, and most probably resulted from an intracellular accumulation of ^{125}I -labelled insulin after inhibition of lysosomal enzymes by chloroquine. To evaluate the intracellular location of the cell-associated insulin in chloroquine-treated cardiac cells, we have studied the displacement of associated ^{125}I -labelled insulin by addition of excess of unlabelled insulin. Some 16% of radioactivity specifically bound after 2 h of incubation was non-dissociable from the control cells by 60 min; this fraction increased about 2.5-fold, to 41%, in chloroquine-treated cells (Fig. 7). This increase in the non-susceptible fraction strongly suggests the intracellular location of the chloroquine-mediated increased radioactivity.

Discussion

It is now generally accepted that the interaction of insulin with a target cell represents a complex phenomenon involving a number of different processes. Recent studies on isolated cells and perfused organs have provided new insights into the fate of insulin after the initial binding event. However, the different steps after receptor binding are still poorly understood, and it is not clear whether insulin is processed by a common pathway by different cell types.

Isolated cardiac myocytes, as used in the present investigation, provide an excellent model for studies on the function and metabolic regulation of cardiac muscle (Vahouny *et al.*, 1979; Farmer *et al.*, 1977; Altschuld *et al.*, 1980). The characteristics of insulin-receptor interaction in this system have been described in reports from our laboratory (Eckel & Reinauer, 1980a,b; Eckel *et al.*, 1982). In the present investigation attempts have been made to elucidate the pathways involved in insulin metabolism after the initial binding event.

Steady-state conditions of insulin binding at 37°C have been established by a modification of our previously published procedures. Under these conditions no tendency for compartmentalization or irreversible binding of insulin could be detected. These data are in agreement with the observations of Olefsky *et al.* (1979) for insulin binding to isolated adipocytes, but contrast with a report by Kahn & Baird (1978), who suggested that the hormone is rapidly transferred into a 'compartment' which is less accessible from the external milieu. Our data suggest that all the receptors are maintained in equilibrium with extracellular insulin regardless of the state of the insulin-receptor complex. This, however, does not exclude the possibility of internalization and intracellular degradation of receptor-

bound hormone (see below), since this process may proceed very rapidly compared with the association–dissociation reaction.

Under steady-state conditions 96% of specifically bound radioactivity consisted of intact insulin, whereas 4% was represented by low-molecular-weight material. This fraction remained unaltered up to 180 min of incubation, suggesting that cardiac myocytes do not accumulate the fragments formed by insulin degradation, as shown for the isolated adipocyte (Gliemann & Sonne, 1978; Kahn & Baird, 1978). On the other hand, a time-dependent increase in the myocyte-associated V_0 material could be detected. This high-molecular-weight material has been thought to represent a physiologically important association of insulin with its receptor or with other cellular components (Kahn & Baird, 1978). Our observations argue against the physiological relevance of the V_0 material. Firstly, essentially all of this material is non-specifically bound to the cardiocytes; secondly, the formation of this product can be observed in the absence of cells. Most probably this material originates from covalent complexes formed by thiol exchange, as pointed out by other authors (Mahboubi & Smith, 1977; Goldstein & Livingston, 1980).

Increasing evidence is now available that the degradation of insulin by target cells is mediated by both receptor-dependent and receptor-independent pathways (Gliemann & Sonne, 1978; Suzuki & Kono, 1979; Duckworth *et al.*, 1981). In the present investigation we have determined the affinity of the total degrading activity of isolated cardiac myocytes. The K_m value of 1.75×10^{-7} M is about 100 times lower than the affinity of the insulin receptor (Eckel & Reinauer, 1981), suggesting the presence of a receptor-independent pathway for insulin degradation. Most likely, this may be due to an insulin-specific proteinase ('insulinase') as shown for muscle and liver tissue (Brush, 1971; Burghen *et al.*, 1972). The physiological implications of this enzyme for the mechanism of insulin action are unclear at the present time, though it is known to account for most of the degradation of the hormone (Suzuki & Kono, 1979). From our data it can be calculated that 70–80% of cardiac insulin degradation is mediated by such a proteinase.

A second pathway of cardiac insulin metabolism involving receptor binding has been identified in the present study. Under steady-state conditions 42% of bound insulin dissociates in a degraded form, whereas very early in the association phase this fraction decreases to less than 10%. These data suggest that the insulin–receptor complex is translocated or processed within the plasma membrane, and in this way made available for the degradation reaction. Similar observations have been reported for isolated adipocytes (Gliemann & Sonne, 1978;

Olefsky *et al.*, 1979). Under steady-state conditions an appreciable amount (17%) of radioactivity was eluted in the position of the A-chain, suggesting that the receptor-linked degradation of insulin by the isolated heart cell proceeds in a sequential way. Duckworth *et al.* (1981) demonstrated the production of intermediate-sized fragments by hepatocytes in primary culture; however, essentially no intermediate-sized products have been observed in experiments with isolated adipocytes (Kahn & Baird, 1978; Gliemann & Sonne, 1978). It remains to be established if these differences actually reflect a different pattern of insulin metabolism between different cell types or are the result of technical differences between several laboratories.

Receptor-mediated degradation of insulin presumably includes a variety of different steps such as an initial degradation occurring at the membrane level, internalization and degradation by cytoplasmic and lysosomal enzymes. Internalization and lysosomal degradation of insulin have been extensively studied by morphological approaches. Using electron-microscopic radioautography, Carpentier *et al.* (1978) demonstrated that internalized insulin is taken up by lysosomes that are located near the cell surface. Moreover, Goldfine & Smith (1976) reported that up to 50% of labelled insulin may enter the cell under certain conditions. A second, indirect, approach is based on the action of chloroquine, which is a well-documented lysosomotropic inhibitor of proteolysis. In the present investigation we have studied the effect of chloroquine on the interaction of insulin with cardiac myocytes. After treatment of cells the non-dissociable fraction increased by 250%, whereas the receptor-linked degradation was significantly decreased. These data suggest internalization and lysosomal processing of insulin by the isolated heart cell. The physiological meaning of this process is still unclear; however, since it accounts for only 20–30% of total insulin degradation, it may be of minor importance for insulin metabolism. On the other hand, it may be speculated that after internalization the hormone is degraded to release an active fragment mediating some intracellular response (Goldfine, 1977).

For the first time we have now demonstrated an active metabolism of insulin in the heart muscle which is comparable with that in other target tissues such as fat and liver. The implications of this metabolism for insulin action under normal and pathological conditions remain to be elucidated.

The excellent technical assistance of Miss Gabriele Traxel is gratefully acknowledged. This work was supported by the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen, the Bundesministerium für Jugend, Familie und Gesundheit and the Deutsche Forschungsgemeinschaft (SFB 113).

References

- Altschuld, R., Gibb, L., Ansel, A., Hohl, C., Kruger, F. A. & Brierley, G. P. (1980) *J. Mol. Cell. Cardiol.* **12**, 1383–1395
- Bergeron, J. J. M., Posner, B. I., Josefsberg, Z. & Sikstrom, R. (1978) *J. Biol. Chem.* **253**, 4058–4066
- Brush, J. S. (1971) *Diabetes* **20**, 151–155
- Burghen, G. A., Kitabchi, A. F. & Brush, J. S. (1972) *Endocrinology* **91**, 633–642
- Carpentier, J. L., Gorden, P., Amherdt, M., Van Obberghen, E., Kahn, C. R. & Orci, L. (1978) *J. Clin. Invest.* **61**, 1057–1070
- Carpentier, J. L., Gorden, P., Freychet, P., Le Cam, A. & Orci, L. (1979a) *J. Clin. Invest.* **63**, 1249–1261
- Carpentier, J. L., Gorden, P., Barazzzone, P., Freychet, P., Le Cam, A. & Orci, L. (1979b) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2803–2807
- Chain, E. B., Mansford, K. R. L. & Opie, L. H. (1969) *Biochem. J.* **115**, 537–546
- Cheung, J. Y., Conover, C., Regen, D. M., Whitfield, C. F. & Morgan, H. E. (1978) *Am. J. Physiol.* **234**, E70–E78
- De Duve, C., De Barse, T., Poole, B., Trouet, A., Tulkens, P. & Van Hoof, F. (1974) *Biochem. Pharmacol.* **23**, 2495–2531
- Duckworth, W. C. (1978) *Endocrinology* **102**, 1766–1774
- Duckworth, W. C., Runyan, K. R., Wright, R. K., Halban, P. A. & Solomon, S. S. (1981) *Endocrinology* **108**, 1142–1147
- Eckel, J. & Reinauer, H. (1980a) *Biochim. Biophys. Acta* **629**, 510–521
- Eckel, J. & Reinauer, H. (1980b) *Biochem. Biophys. Res. Commun.* **92**, 1403–1408
- Eckel, J. & Reinauer, H. (1981) *IRCS Med. Sci.* **9**, 863–864
- Eckel, J., Offermann, A. & Reinauer, H. (1982) *Basic Res. Cardiol.* in the press
- Farmer, B. B., Harris, R. A., Jolly, W. W., Hathaway, D. R., Katzberg, A., Watanabe, A. M., Whitlow, A. L. & Besch, H. R. Jr. (1977) *Arch. Biochem. Biophys.* **179**, 545–558
- Fischer, U. (1971) *Acta Biol. Med. Germ.* **26**, 87–99
- Freychet, P., Kahn, R., Roth, J. & Neville, D. M., Jr. (1972) *J. Biol. Chem.* **247**, 3953–3961
- Gliemann, J. & Sonne, O. (1978) *J. Biol. Chem.* **253**, 7857–7863
- Goldfine, I. D. (1977) *Diabetes* **26**, 148–155
- Goldfine, I. D. & Smith, G. J. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1427–1431
- Goldstein, B. J. & Livingston, J. N. (1980) *Biochem. J.* **186**, 351–360
- Gorden, P., Carpentier, J. L., Freychet, P. & Orci, L. (1980) *Diabetologia* **18**, 263–274
- Hammond, J. M. & Jarret, C. (1975) *Diabetes* **24**, 1011–1019
- Kahn, C. R. (1976) *J. Cell Biol.* **70**, 261–286
- Kahn, C. R. & Baird, K. (1978) *J. Biol. Chem.* **253**, 4900–4906
- Kones, R. J. & Phillips, J. H. (1975) *Cardiology* **60**, 280–303
- Kono, T., Robinson, F. W., Sarver, J. A., Vega, F. W. & Pointer, R. H. (1977) *J. Biol. Chem.* **252**, 2226–2233
- Le Cam, A., Freychet, P. & Lenoir, P. (1975) *Diabetes* **24**, 566–573
- Mahboub, M. & Smith, H. J. (1977) in *Protein Crosslinking* (Friedman, M., ed.), pp. 247–260. Plenum Press, New York
- Marshall, S. & Olefsky, J. M. (1979) *J. Biol. Chem.* **254**, 10153–10160
- Morgan, H. E., Randle, P. J. & Regen, D. M. (1959) *Biochem. J.* **73**, 573–579
- Olefsky, J. M., Kobayashi, M. & Chang, H. (1979) *Diabetes* **28**, 460–471
- Powers, A. C., Solomon, S. S. & Duckworth, W. C. (1980) *Diabetes* **29**, 27–32
- Sonne, O. & Gliemann, J. (1980) *J. Biol. Chem.* **255**, 7449–7454
- Suzuki, K. & Kono, T. (1979) *J. Biol. Chem.* **254**, 9786–9794
- Terris, S. & Steiner, D. F. (1975) *J. Biol. Chem.* **250**, 8389–8398
- Tsai, J. S. & Seeman, M. (1981) *Biochim. Biophys. Acta* **673**, 259–269
- Vahouny, G. V., Wei, R. W., Tamboli, A. & Albert, E. N. (1979) *J. Mol. Cell. Cardiol.* **11**, 339–357
- Wibo, M. & Poole, B. (1974) *J. Cell Biol.* **63**, 430–440
- Yokono, K., Imamura, Y., Sakai, H. & Baba, S. (1979) *Diabetes* **28**, 810–817