Diabetologia (1988) 31: 315-321

Diabetologia

Plasma membrane phospholipid content in non-insulin-dependent streptozotocin-diabetic rats - effect of insulin

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Summary. The activity of $(Ca^{2-} + Mg^{2+})$-ATPase is impaired in kidney basolateral membranes from non-insulin-dependent streptozotocin-diabetic rats. To study the possible role of changes in membrane phospholipid content in the malfunction of this enzyme in kidney membranes of the diabetic animals, phospholipid (phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and sphingomyelin) content was measured in kidney and liver membranes obtained from non-insulin-dependent diabetic rats. Total phospholipid content was similar in liver and kidney membranes of diabetic and control rats (595 ± 47 versus 624 ± 29 in liver and 469 ± 22 versus 458 ± 17 nmol Pi/mg protein in kidney respectively). Phosphatidylethanolamine content in kidney and liver membranes of diabetic rats was lower than in control rats (87.7 ± 1.8 versus 96.4 ± 2.2 nmol Pi/mg protein, $p < 0.01$ and 87.1 ± 3.7 versus 101.8 ± 3.5, $p < 0.02$ respectively). Phosphatidylinositol content was higher in kidney (28.0 ± 0.6 versus 23.9 ± 2.1, $p < 0.02$) but not liver membranes from diabetic rats.

The in vitro direct effect of insulin on the phospholipid content in kidney membranes was also measured. Physiologic concentrations of insulin (718 pmol/l for 30 min) increased the phosphatidic acid content in membranes from control but not from diabetic rats by 34.2% ($p < 0.02$). This rise was readily measurable after 3 min of exposure to insulin. Insulin did not induce a significant change in the content of any other phospholipid in membranes from control or diabetic rats.

These differences in phospholipid content demonstrated in isolated membranes obtained from non-insulin-dependent diabetic and control rats, before and after exposure to insulin, may explain, in part, the impaired function of the $(Ca^{2+} + Mg^{2+})$-ATPase observed previously in kidney membranes of the diabetic rats.

Key words: Phospholipids, membrane, diabetes, insulin.

A variety of polypeptide hormones can provoke rapid changes in phospholipid metabolism in their target tissues [1-7]. Insulin acutely increases phospholipids in the phosphatidate-inositide cycle in rat adipose tissue both in vivo and in vitro [2, 8]. Shifts in membrane phospholipid content may be important in regulating the activity of a variety of cellular enzymes, including those which control intracellular Ca^{2+} homeostasis. In this regard, purified $(Ca^{2+} + Mg^{2+})$-ATPase can be regulated by a variety of acidic phospholipids [9]. Since it is clear that this enzyme requires phospholipids for its activity within the plasma membrane [10, 11], changes in membrane phospholipid content would be expected to affect its activity.

Neonatal rats rendered diabetic by streptozotocin (STZ) injection develop non-insulin-dependent diabetes and have impaired peripheral insulin action in vivo and in vitro [12-15]. We have shown that exposure to physiologic concentrations of insulin increased the $(Ca^{2+} + Mg^{2+})$-ATPase activity in kidney basolateral membranes (BLM) obtained from normal dogs and rats [16, 17]. However, in BLM obtained from the diabetic rats the basal $(Ca^{2+} + Mg^{2+})$-ATPase activity was higher than in control rats and failed to respond to insulin in vitro [16].

We propose that changes in plasma membrane phospholipid composition may play a significant role in the impairment of $(Ca^{2+} + Mg^{2+})$-ATPase activity observed in BLM of diabetic rats [16]. To investigate this possibility, we have studied phospholipid content in kidney BLM obtained from control and diabetic rats as well as the direct in vitro effect of insulin on phospholipid content of these membranes. In order to assess whether changes in membrane phospholipid composition in diabetic rats are limited to kidney BLM, we have also evaluated the composition of phos-

pholipid content in liver membranes obtained from these non-insulin-dependent diabetic and control rats.

Materials and methods

Two-day-old Sprague-Dawley rat pups were injected i.p. with 90 mg/kg body weight STZ (Upjohn Co., Kalamazoo, Mich, USA) in 0.1 mol/l citrate buffer, pH 4.5 according to previously described methods [14-16]. At 4 days of age 0.1 ml of blood was collected into heparinised capillary tubes by cardiac puncture and plasma glucose was measured by the glucose-oxidase method. Animals that demonstrated plasma glucose levels of 11.2 nmol/l or above were selected as diabetic [12, 18]. The animals were weaned at 24 days of age and separated according to sex. Only male rats were used in this study. The rats were kept at 4-5 animals per cage, exposed to 10-h on, 14-h off light cycles and were allowed to feed ad libitum on standard rat laboratory chow (Purina 5001). At age 11 weeks the animals were weighed and then killed by decapitation. The animals were in the non-fasting state and all decapitations were performed between 09.00-11.00 hours. To minimize the effect of time of killing on the results, diabetic and control animals were killed in random order until all the animals were used.

Blood specimens were collected from the decapitated rats directly into heparinised 50 ml plastic tubes and after separation the plasma was kept frozen at −20 °C for glucose and insulin determinations. The livers and kidneys were immediately separated, weighed and kept on ice for membrane preparations.

Membrane preparations

Suspensions enriched for kidney BLM originating from kidney cortical mince were prepared according to the method of Sacktor et al. [19] using a Percoll gradient ultracentrifugation [20]. The final two centrifugations of the preparative procedure for BLM preparation were performed using a 0.25 mol/l sucrose, 10 mmol/l $MnCl_2$, 50 mmol/l Tris HCl, pH 7.5 solution [16]. Ten different membranes were prepared from each group (diabetic rats and controls); kidneys from 7-8 animals were used for each membrane preparation. Liver membranes were prepared from 6 different individual animals (diabetic rats and controls) using the method of Tsushima and Friesen [21]. Protein determination was done by the method of Lowry [22]. After preparation the membranes were separated into aliquots containing 1 mg protein samples and kept frozen at −70 °C until phospholipid measurements were performed. As previously described in detail [16], these kidney BLM membranes show 10-11-fold enrichment in the plasma membrane marker, $(Na^+ + K^+)$-ATPase compared to homogenates. Membranes from the diabetic and control animals had the same degree of purification.

Glucose was measured using the Beckman Glucoanalyzer II (Beckman Instruments, Fullerton, Calif, USA) [23]. Insulin was determined by the radioimmunoassay method of Morgan and Lazarow [24]. Coefficient of variation of the technique was 6.5%.

In vitro hormone treatment

1.5 mg protein of kidney BLM obtained from control and diabetic rats were incubated in a medium containing 5.5 mmol/l glucose, 2 μmol/l Ca^{2+} [16], 120 mmol/l Tris HCl, pH 7.2, at 37 °C, with and without insulin (718 pmol/l). Incubation time was 30 min unless otherwise specified. Incubation was terminated by addition of ice cold chloroform-methanol solution (1:2 v/v) and phospholipids were immediately extracted and measured.

Phospholipid determination

Membrane phospholipids were extracted by the method of Bligh & Dyer [25]. The final chloroform lipid phase was stored under N_2 at −80 °C in microvials, coated with Glass Treet (Alltech Associates, Inc., Deerfield, Ill, USA). The extract was evaporated under a stream of N_2 and resuspended in 100 μl of chloroform: methanol (1:1) and subjected to thin layer chromatography. For one dimensional separation, samples were spotted on pre-coated Silica-Gel 60 plate (E. Merck, Darmstadt, FRG) and chromatographed in chloroform: methanol: acetic acid: distilled water (100:30:35:3, v/v). This solvent system separates sphingomyelin (Spm), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidic acid (PA), and cardiolipin (RF) values approximately 0.08, 0.17, 0.33, 0.44, 0.60, 0.85, and 0.90, respectively [26]. For two dimensional separations, two different solvent solutions were used. The first solvent system consisted of chloroform: methanol: concentrated ammonium hydroxide (15 mol/l): water (65:35:5:0.6, v/v) while the second solvent system comprised chloroform: methanol: glacial acetic acid: water (80:24:38:3.5, v/v). After development, the plates were dried and phospholipid spots were visualised by iodine vapor and identified by comparison to migration of authentic standards (Sigma Chemical, St. Louis, MO, USA). The spots were scraped from the plates and digested in 10 N H_2SO_4 for 3 h at 160 °C. Then 4 drops of 30% hydrogen peroxide were added and samples were reheated for 1.5 h at 160 °C. Phosphorus content of the digest was determined by the method of Bartlett [27]. The membrane phospholipid content was expressed as nmol Pi/mg protein. Total lipid phosphorus was determined directly from the lipid extract by the method described above, and results were calculated as nmol Pi/mg protein. Two dimensional chromatography for separation of the phospholipids was used in the incubation studies to assure that contamination with new phospholipid metabolites did not influence the measurements of the phospholipids of interest in this study. All phospholipid assays were done in duplicates.

Statistical analysis

Results were expressed as mean ± SEM. Statistics for the determination of the membrane phospholipid content were performed using two tailed t-test. Results from the incubation experiments were analysed using Student's paired t-test, since direct comparisons were made on the same membrane preparations under identical conditions except for the presence of insulin.

Results

The characteristics of the diabetic and control animals used for the liver and kidney membranes preparations are given separately in Tables 1 and 2, respectively, since two separate paired groups of animals were used for these procedures. The weight of the diabetic animals at the time of killing was lower by 9% compared to control rats for both sets of animals ($p < 0.05$ and

Table 1. Rats used for liver membrane preparations

	Glucose (mmol/l)	Insulin (pmol/l)	Body weight (g)	Liver weight (% of body weight)
Control rats (6)	7.84 ± 0.17	120.6 ± 8.9	229.3 ± 7.1	5.5 ± 0.5
Diabetic rats (6)	10.30 ± 0.73[b]	143.6 ± 0.73	199.0 ± 10.6[a]	5.3 ± 0.3

Results are given as mean ± SEM. The number of rats is given in parentheses. [a] $p < 0.05$; [b] $p < 0.01$ diabetic versus control rats

$p < 0.001$, respectively). There was no difference in the kidney or liver weight between the diabetic and control rats when these values were expressed as percentage of total body weight (Tables 1 and 2).

There was no difference between the diabetic and control rats in the random plasma insulin values. Non-fasting plasma glucose values obtained at the same time were significantly higher in the diabetic rats ($p < 0.01$-$p < 0.001$). The lack of difference in plasma insulin values between the diabetic and control animals is consistent with our previous report [19] where the random plasma insulin levels of the diabetic rats were only slightly decreased and averaged 73% of the control rats.

The results describing the phospholipid content in kidney BLM from the diabetic and control animals are given in Table 3. BLM obtained from the diabetic rats revealed significantly higher PI content and lower PE content ($p < 0.02$ and $p < 0.01$, respectively). In addition, the crude liver membranes obtained from the diabetic rats also revealed a lower PE content (Table 4). There was a marked overall difference in the phospholipid content of the liver membranes compared to that of the kidney BLM (Tables 3 and 4). Total phospholipid content of the liver membranes and kidney BLM obtained from control rats was not different from that in the membranes obtained from the diabetic animals (595 ± 47 vs 624 ± 29 and 469 ± 22 vs 458 ± 17 nmol Pi/mg protein respectively).

The effects of in vitro insulin treatment of BLM on phospholipid content were then examined. The incubation conditions by themselves did not result in a significant change in total phospholipid content. The levels found before and after incubations in the absence of insulin were 469 ± 22 vs 448 ± 37 for control rats and 458 ± 17 vs 478 ± 49 nmol Pi/mg protein for diabetic rats respectively. After incubation of the membranes, the difference in PI and PE content between BLM of control and diabetic rats did not reach significance, possibly due to increased variability (and SEM) in the phospholipid values of the incubated membranes.

Upon addition of insulin to the in vitro incubations no significant changes in total phospholipid content of BLM were noted for control (452 ± 37 nmol Pi/mg protein) or for diabetic rats (487 ± 56 nmol Pi/mg protein). However, there was a measurable effect of insulin on PA content in BLM of control rats (Table 5). This effect was time-dependent. After a 3-min incubation of control BLM with physiological concentrations of insulin (718 pmol/l), there was a $26.7 \pm 5.5\%$ increase in PA, which progressed to 32.0 ± 6.7 by 10 min and remained elevated for 30 min (Table 6). By 60 min, PA levels had returned to baseline. Insulin did not change significantly the content of the other phospholipids measured (PE, PS, PI, PC, Spm) in membranes

Table 2. Rats used for kidney basolateral preparations

	Glucose (mmol/l)	Insulin (pmol/l)	Body weight (g)	Kidney weight (% of body weight)
Control rats (64)	7.84 ± 0.11	155.1 ± 16.7	232 ± 6	0.74 ± 0.2
Diabetic rats (61)	11.25 ± 0.67^b	143.6 ± 10.8	212 ± 4^a	0.74 ± 0.3

Results are given as mean ± SEM. The number of animals is given in parentheses. [a] $p < 0.02$; [b] $p < 0.001$ diabetic versus control rats

Table 3. Phospholipid composition in kidney basolateral membranes of diabetic and control rats

	Phospholipids					
	PA	PE	PS	PI	PC	Spm
	(nmol Pi/mg protein)					
Diabetic rats (8)	8.02 ± 0.6	87.7 ± 1.8^b	38.2 ± 3.1	28.0 ± 0.6^a	138.9 ± 6.9	75.4 ± 1.8
Control rats (8)	8.1 ± 0.6	96.4 ± 2.2	36.7 ± 2.1	23.9 ± 2.1	136.9 ± 5.9	74.7 ± 2.4

PA - phosphatidic acid; PE - phosphatidylethanolamine; PS - phosphatidylserine; PI - phosphatidylinositol; Spm - sphingomyelin; PC - phosphatidylcholine. Numbers in parentheses refer to number of membranes used. Each membrane preparation was from 7-8 animals. Results are given as mean ± SEM. Statistics were done using two tailed t-test. [a] $p < 0.02$; [b] $p < 0.01$ diabetic versus control rats

Table 4. Phospholipid composition in liver membranes of diabetic and control rats

	Phospholipids					
	PA	PE	PS	PI	PC	Spm
	(nmol Pi/mg protein)					
Diabetic rats (6)	10.0 ± 1.3	87.1 ± 3.7^a	11.9 ± 1.2	40.4 ± 1.3	312.8 ± 17.8	19.5 ± 1.5
Control rats (6)	9.3 ± 0.5	101.8 ± 3.5	13.1 ± 0.7	41.6 ± 3.6	313 ± 12.8	21.9 ± 1.4

The numbers in parentheses refer to the number of membranes used. Results are given as mean ± SEM. Statistics were done using two tailed t-test. [a] $p < 0.02$

Table 5. Effect of insulin on phospholipid composition in kidney basolateral membranes from diabetic and control rats

	Phospholipids					
	PA	PE	PS	PI	PC	Spm
	(nmol Pi/mg protein)					
BLM of diabetic rats in presence of insulin (10)	7.0 ± 0.6	92.3 ± 7.2	37.4 ± 4.4	28.2 ± 3.6	151.0 ± 9.8	69.3 ± 7.6
BLM of diabetic rats in absence of insulin (10)	6.9 ± 0.5	91.3 ± 5.3	37.0 ± 3.4	28.06 ± 2.6	153.7 ± 11.4	72.5 ± 6.1
BLM of control rats in presence of insulin (10)	8.2 ± 0.6[a]	96.9 ± 5.1	33.2 ± 5.1	24.7 ± 3.7	139.5 ± 8.5	68.0 ± 6.8
BLM of control rats in absence of insulin (10)	6.1 ± 0.7	97.9 ± 7.5	33.2 ± 3.8	25.1 ± 2.9	137.2 ± 7.9	65.5 ± 7.0

Basolateral membranes - BLM incubation time was 30 min. Numbers in parentheses are number of membranes used. Each membrane preparation was from 7-8 animals. Results are given as mean ± SEM. Comparison was done between membranes (from diabetic and control rats) incubated with and without insulin. Statistics were done using paired Student's t-test [a] $p < 0.02$ versus values of membranes from control rats in the absence of insulin

Table 6. Insulin-induced changes in phosphatidic acid content with time in kidney basolateral membranes of control and diabetic rats

	Type of rat	Time (min)			
		3	10	30	60
Increase in PA content (nmol Pi/mg protein)	Control (4)	2.0 ± 0.3[a]	2.3 ± 0.3[a]	2.1 ± 0.3[a]	0.4 ± 0.4
Increase in PA content (percent over baseline)	Control (4)	26.7 ± 5.5[a]	32.0 ± 6.7[a]	34.2 ± 7.4[a]	5.7 ± 7.2
Increase in PA content (nmol Pi/mg protein)	Diabetic (4)	0.7 ± 0.4	0.4 ± 0.3	0.2 ± 0.4	0.4 ± 0.4
Increase in PA content (percent over baseline)	Diabetic (4)	9.3 ± 6.2	6.1 ± 4.8	2.8 ± 5.4	4.1 ± 6.0

Increase = difference in PA content in presence and absence of insulin in the incubation medium. Baseline = PA content in the absence of insulin. Comparison was done between increases in PA content in membranes of control and diabetic rats using two-tailed t-test. Number of membranes used is indicated in parentheses. Results are given as mean ± SEM. [a] $p < 0.05$ when increases in PA content in control rat membranes are compared to increases in PA in membranes of diabetic rats

from both diabetic and control animals, either after 30 min (Table 5) or after 3, 10 and 60 min of incubation (data not shown). In membranes from diabetic rats no significant insulin effect on PA content was observed either after 30 min (Table 5) or after any of the other time point studied (Table 6). In membranes from control rats only, total PA content in the presence of insulin was higher than those in the absence of the hormone at all time points ($p < 0.02$-$p < 0.05$ when evaluated by paired Student's t-test). Data for 30 min only are given in Table 5.

Discussion

Abnormal $(Ca^{2+} + Mg^{2+})$-ATPase activity in membranes from non-insulin-dependent diabetic rats has been suggested as contributing to the impaired insulin action seen in these animals [12, 13, 16]. Liver and kidney cells contain specific insulin receptors [28-31], $(Ca^{2+} + Mg^{2+})$-ATPase activity [16, 32] and are known sites for insulin action [33, 34]. Therefore, plasma membranes obtained from these tissues were studied to investigate a possible role of membrane phospholipid in the abnormal activity of $(Ca^{2+} + Mg^{2+})$-ATPase in the diabetic rats. Liver and kidney membranes obtained from the diabetic animals revealed decreased PE content. Increased PI content was seen only in kidney BLM from the diabetic rats. These observations and the different overall pattern of the phospholipids in liver and kidney membranes (Tables 3 and 4) reveal that there is a tissue difference in the membrane phospholipid content. However, the diabetic state was associated with at least one change in the phospholipid content of plasma membranes common to both tissues, a relative decrease in PE. This change could not be attributed to a difference in diet between the two groups of animals since both were fed the same rodent chow and have been shown to consume the same amount of food [18].

Sauerheber et al. [35], who studied membrane lipid structure in adipocytes from control and STZ-treated diabetic rats using spin label techniques, could not show a difference between the two animal groups. This finding led the authors to conclude that no gross alteration in the membrane lipid structure occurred in cells from diabetic animals. However, the authors admitted that regional changes in membrane lipid composition could exist which they would not be able to detect by their technique. Thus, the small but significant changes observed by us using isolation and separation techniques could easily be missed. The magnitude of difference in PE and PI content in membranes from the diabetic rats compared with their controls is similar to that reported earlier in platelet membranes of diabetic patients and control subjects [36]. In that study, membranes from diabetic patients differed from control subjects in their PS and PE content. Expressed as per-

centage of total phospholipid, membrane PS and PE contents were 5.5 ± 0.7 (mean $\pm$ SD) vs 6.3 ± 0.5 (PS) and 27.2 ± 2.7 vs 29.2 ± 3.0 (PE) in control subjects and diabetic patients respectively. These differences were highly significant ($p < 0.001$ and $p < 0.01$). This magnitude of difference suggests that the disease condition is associated with only a small change in phospholipid content, affecting mainly those phospholipids which predominate the inner half of the membrane lipid bilayer, PE, PS, PI [37, 38].

According to the proposed scheme of Carafoli [39], the $(Ca^{2+} + Mg^{2+})$-ATPase molecule is so oriented that its active part is on the inner side of the membrane. It is the immediate phospholipid milieu surrounding the $(Ca^{2+} + Mg^{2+})$-ATPase molecule that exerts the greatest influence on the enzyme activity [10]. Thus, the different PE and PI content in BLM of the non-insulin-dependent animals could account for the abnormal $(Ca^{2+} + Mg^{2+})$-ATPase activity in these membranes [16], and a corresponding lack of insulin-regulated enzyme activity. Recently, Wali et al. [40] reported increased accessibility of PE and PS to phospholipase A_2 of erythrocytes of diabetic patients. The authors suggested that loss of the asymmetry in distribution of phospholipids between the inner and outer membrane layers could account for this pathology. Whether similar changes occur in the BLM of the diabetic rats and further magnify the effect of the change observed in their PI and PE contents remains to be determined.

Of special interest is the finding that insulin induced a significant increment (approximately 33%) in PA content only in membranes from control rats with no effects noted in BLM from diabetic rats (Table 5 and 6). Acidic phospholipids increase $(Ca^{2+} + Mg^{2+})$-ATPase activity [9]. Consequently, it is possible that the increase in PA content in membranes from control rats could mediate the stimulating effect of insulin on the enzyme activity [16, 17]. Similarly, a lack of increase in PA in membranes from the diabetic rats may be important in explaining the lack of regulatory effect of the hormone on $(Ca^{2+} + Mg^{2+})$-ATPase in membranes from the diabetic rats [16]. The currently described changes in membrane PA content induced by insulin are much smaller than those previously used by Niggli et al. [9] to induce an increase in the activity of the enzyme. However, while in that study a purified enzyme embedded in an artificial micelles of Triton X-100 and phosphatidylcholine was studied, our observations of insulin-induced increase in both the $(Ca^{2+} + Mg^{2+})$-ATPase activity [16] and PA content occurred in intact membranes. It is, therefore, possible that under more physiologic conditions (intact membranes) much smaller quantities of acidic phospholipids are sufficient to increase the enzyme activity. Furthermore, in the study of Niggli the increases in the ATPase activity induced by acidic phospholipids were up to 200-400% over basal while insulin-increase in the enzyme activity in control membranes previously observed by us was in the range of 30-35% over basal [16]. Thus, it is possible that the currently observed insulin-induced changes in PA content could explain, in part, its regulatory effect on the enzyme.

The cause for the lack of effect of insulin on PA content in membranes of diabetic animals is not clear. Insulin binding is not decreased in BLM of diabetic rats [16]. Thus, the lack of insulin effect on PA content in these membranes cannot be attributed to decreased insulin binding to its receptor. It is possible that more complex redistribution of the different phospholipids in the membrane bilayers occurs in the membranes of the diabetic rats [40]. As a result of this redistribution and the observed changes in phospholipid content, insulin might lose its ability to increase the PA content in these membranes.

The sequence of events suggested by our results predicts that insulin-induced changes in phospholipids precede the changes in $(Ca^{2+} + Mg^{2+})$-ATPase activity induced by the hormone. In the current study insulin had already increased PA content in control membranes (27%) after 3 min (Table 6). In fact, it is likely that these changes start even earlier. Recently, it was reported that insulin induced a 20% increase in PA content in BC3Hl myocytes after only 30 s [41]. Therefore, the rise in the membrane PA content could conceivably mediate the stimulatory effect of insulin on $(Ca^{2+} + Mg^{2+})$-ATPase activity. After 60 min, the insulin-induced increase in membrane PA disappeared and PA content returned to baseline. Whether this occurred by direct conversion of PA into diacylglycerol as recently suggested to occur in myocytes [42], cannot be answered since diacylglycerol was not measured.

The mechanism by which insulin caused the increase in membrane PA remains unclear, although a very likely site of such action is at the level of plasma membrane phospholipase activity. While a direct effect of insulin on membrane phospholipase was not demonstrated, this must be considered a strong possibility. The increase in PA content was not accompanied by a detectable decrease in any other specific phospholipid. This suggests that more than one phospholipid precursor contributed to the increase in membrane PA, as would occur by phospholipase D action.

Phospholipase D activity was demonstrated in several rat tissues including kidney [43]. Further, it has been suggested that the insulin-mediated increase in PA content in myocytes could be generated directly by phospholipase D [41]. Studies designed to investigate a direct regulatory effect of insulin on phospholipase D activity are needed to confirm this possibility.

The possibility that the changes observed in $(Ca^{2+} + Mg^{2+})$-ATPase activity [16] and membrane phospholipid content in BLM of diabetic rats were due to toxic effects of STZ can be raised. However, several lines of evidence argue against it. These include: 1) In the diabetic animals kidney function and histology

were shown to be normal [18] and the activity of another ATPase, $(Na^+ + K^+)$-ATPase, in their kidney BLM is not altered [44]; 2) The previously described loss of ability of insulin to regulate the $(Ca^{2+} + Mg^{2+})$-ATPase activity in kidney BLM of diabetic rats could be reversed by physiologic perturbation (submitting the animals to an 18-h food restriction) [45]; 3) Abnormalities in $(Ca^{2+} + Mg^{2+})$-ATPase were recently observed by us in erythrocyte membranes of diabetic animals (unpublished data) and by others [46], in erythrocytes of diabetic patients [46] revealing that the defect in this enzyme activity in diabetes is not restricted to kidney tissue and can occur in the absence of STZ; 4) Similar to our observations, alterations in membrane phospholipids content and behaviour (specifically PE) were described also in erythrocytes and platelets of diabetic patients [36, 40]. Taken together, it appears that the possibility that our observations in the diabetic animals are due to toxic effects of STZ is unlikely.

In conclusion, the present study reveals that BLM from non-insulin-dependent streptozotocin-diabetic rats have abnormal phospholipid content which could account for their impaired $(Ca^{2+} + Mg^{2+})$-ATPase activity. Insulin specifically increases PA content only in normal rat kidney BLM, but not in membranes from the diabetic rats. It is suggested that the acute increase in this acidic phospholipid could explain in part the previously documented stimulatory effect of the hormone on membrane $(Ca^{2+} + Mg^{2+})$-ATPase only in control rats. The precise mechanism by which insulin induces the increase in membrane PA content remains to be investigated.

Acknowledgements. This work was supported by research grants AM20579, AM27600, and AM32087 and by a Research and Career Development Award from the American Diabetes Association.

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Received: 24 August 1987
and in revised form: 12 February 1988

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