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Glucose Rapidly Decreases Plasma Membrane GLUT4 Content in Rat Skeletal Muscle

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We have previously demonstrated that chronic hyperglycemia *per se* decreases GLUT4 glucose transporter expression and plasma membrane content in mildly streptozotocin- (STZ) diabetic rats (*Biochem. J.* 284, 341–348, 1992). In the present study, we investigated the effect of an acute rise in glycemia on muscle GLUT4 and GLUT1 protein contents in the plasma membrane, in the absence of insulin elevation. Four experimental groups of rats were analyzed in the postabsorptive state:

1. **Control rats.**
2. **Hyperglycemic STZ-diabetic rats with moderately reduced fasting insulin levels.**
3. **STZ-diabetic rats made normoglycemic with phlorizin treatment.**
4. **Phlorizin-treated (normoglycemic) STZ-diabetic rats infused with glucose for 40 min.**

The uniqueness of the latter model is that glycemia can be rapidly raised without any concomitant increase in plasma insulin levels. Plasma membranes were isolated from hindlimb muscle and GLUT1 and GLUT4 proteins amounts determined by Western blot analysis. As predicted, STZ-diabetes caused a significant decrease in the abundance of GLUT4 in the isolated plasma membranes. Normalization of glycemia for 3 d with phlorizin treatment restored plasma membrane GLUT4 content in muscle of STZ-diabetic rats. A sudden rise in glycemia over a period of 40 min caused the GLUT4 levels in the plasma membrane fraction to decrease to those of nontreated STZ-diabetic rats. In contrast to the GLUT4 transporter, plasma membrane GLUT1 abundance was not changed by the acute glucose challenge. It is concluded that glucose can have regulatory effect by acutely reducing plasma membrane GLUT4 protein contents in rat skeletal muscle. We hypothesize that this glucose-induced downregulation of plasma membrane GLUT4 could represent a protective mechanism against excessive glucose uptake under hyperglycemic conditions accompanied by insulin resistance.

Key Words: Glucose transporter; GLUT1; phlorizin; glycemia; streptozotocin.

Introduction

Glucose transport in skeletal muscle is mediated by the GLUT1 and GLUT4 glucose transporters. Insulin and muscular contraction acutely increase glucose transport in muscle cells by inducing a rapid translocation of GLUT4 transporters from intracellular storage membranes to the cell surface *(1)*. In contrast, the GLUT1 transporter does not appear to be acutely regulated by these stimuli in muscle. Glucose itself can also modulate its own uptake in muscle. The regulatory role of glucose can be particularly demonstrated in experimental models of diabetes, where plasma glucose levels are elevated. In severely hyperglycemic alloxan-diabetic dogs, the muscle metabolic glucose clearance is abnormally low, but this can be partially or fully restored by acute or long-term normalization of hyperglycemia by treatment with phlorizin, an inhibitor of renal glucose reabsorption *(2–4)*. The modulatory effect of glucose on its own uptake has also been demonstrated in pancreatectomized very mild diabetic rats, where the decreased insulin-mediated glucose disposal in vivo can be normalized by restoration of normoglycemia with prolonged phlorizin administration *(5)*. These studies thus imply that the hyperglycemia-related changes in glucose transport may be part of a regulatory response to ambient glucose concentrations.

We have previously reported that the plasma membrane GLUT4 content is decreased in skeletal muscle of hyperglycemic STZ-diabetic animals presenting near-normal fasting insulinemia *(6)*. In this model of fasting hyperglycemia/

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normoinsulinemia, re-establishment of normoglycemia by long-term (3 d) phlorizin treatment fully restored GLUT4 content in the muscle plasma membrane *(7)*. We therefore concluded that chronic hyperglycemia *per se* is responsible, at least in part, for the downregulating effect of diabetes on plasma membrane GLUT4 content in skeletal muscle. However, whether glucose can acutely (<1 h) regulate GLUT4 protein amounts in the plasma membrane when insulin levels are not concurrently increased still remains controversial. Thus, acute (30- to 60-min) increases in glucose levels have been shown to reduce insulin-stimulated glucose uptake and transport in perfused hindquarter muscles of normal (nondiabetic) rats *(8,9)*. Conversely, an acute period (20- to 80-min) of hyperglycemia has previously been shown to increase GLUT4 content in isolated plasma membranes from hindlimb muscles of normal rats *(10)*. The above studies were performed in intact rats, and there are yet no reports on the effects of hyperglycemia on plasma membrane GLUT4 content in diabetic rats.

Thus, the main goal of the present study was to investigate whether an acute increase in glucose levels in vivo could decrease plasma membrane GLUT4 content in skeletal muscle. In order to prevent an increase in insulin levels with acute administration of exogenous glucose, we took advantage of the phlorizin-treated streptozotocin- (STZ) diabetic rat model, in which acute elevations in blood glucose levels can be achieved without concomitant rises in insulin concentrations. The results show that a sudden (40-min) rise in glycemia decreases GLUT4 content in the plasma membrane of skeletal muscle in diabetic rats.

Results

When compared to control untreated rats, 7-d STZ-treated diabetic rats were markedly hyperglycemic with fasting blood glucose of >300 mg/dL (Table 1). Phlorizin treatment of STZ-diabetic rats for 3 d completely reversed the hyperglycemic effects of STZ, lowering blood glucose to normal levels. Subsequent iv glucose injection for 40 min generated a state of sudden hyperglycemia. Insulin levels were moderately decreased in STZ-diabetic and phlorizin-treated STZ-diabetic rats compared to control rats. Importantly, insulin was not increased by the 40 min glucose administration when compared to phlorizin-treated STZ-diabetic rats not infused with glucose. Glucagon levels were augmented in STZ-diabetic rats and further increased in phlorizin-treated diabetic rats. Sudden re-establishment of hyperglycemia in phlorizin-treated STZ-diabetic rats brought back glucagon to untreated STZ-diabetic rat values.

We have previously reported that the induction of diabetes by the same STZ treatment used in the present study does not alter the characteristics of the membrane fractions isolated from rat skeletal muscle *(6)*. The effects of phlorizin or glucose infusion on the isolation of skeletal muscle plasma membrane fractions were assessed by measuring the protein yield and the enzymatic activity of 5'-nucleotidase, a well-known plasma membrane marker. There were no significant differences in either the protein yields or the activity of 5'-nucleotidase between any of the experimental groups (Table 2). The enzymatic activity of 5'-nucleotidase was enriched by fivefold in the isolated plasma membrane fractions compared to crude nonfractionated membranes.

We next investigated the effects of chronic and acute hyperglycemia on the membrane contents of the GLUT4 protein. Figure 1 (upper panel) shows a representative Western blot where plasma membrane fractions from five different preparations with muscles of STZ-diabetic rats, phlorizin-treated rats and glucose-infused phlorizin-treated diabetic rats, were loaded on the same gel. Several gels were run with membrane fractions from all the experimental groups, and the immunodetected GLUT4 reactive bands analyzed by scanning densitometry (Fig. 1, lower panel). As seen earlier *(7,11)*, STZ-diabetes caused significant decreases in the abundance of the GLUT4 glucose transporter in the plasma membranes. Restoration of normoglycemia over 3 d by phlorizin administration completely reversed the effect of STZ-diabetes on plasma membrane GLUT4 content, bringing it back to control values. A sudden rise in glycemia over a period of 40 min without change in insulin levels caused the GLUT4 levels in the plasma membrane fraction to decrease to those of nontreated STZ-diabetic rats. The modulatory effects of glycemia on GLUT4 are essentially the same if data were expressed per "total" GLUT4 recovered in the plasma membrane fractions, since the protein yields were similar between the experimental groups (Table 2). On the other hand, no effects of phlorizin treatment or acute hyperglycemia were observed on GLUT4 content in crude unfractionated membranes (relative densitometric units: 9.30 ± 0.72, 9.51 ± 1.45, 10.05 ± 1.36 for DIAB, PHLO, and GLUC groups, respectively).

In contrast to the downregulating effect of acute glucose administration on plasma membrane GLUT4 protein content, the abundance of the GLUT1 glucose transporter in the plasma membrane was not reduced by glucose infusion in phlorizin-treated diabetic rats (Fig. 2). In fact, GLUT1 tended to change in parallel to glycemia and therefore in the opposite direction than that of GLUT4. Thus, GLUT1 content in the plasma membrane was found to be slightly increased in diabetic rats (chronic hyperglycemia) and was returned to control levels by normalizing glycemia for 3 d with phlorizin. However, the acute rise in plasma glucose levels failed to affect plasma membrane GLUT1 to any extent.

Discussion

Previous studies have shown that glycemia is an important determinant of glucose uptake and utilization in skeletal muscle. In the diabetic state, chronic hyperglycemia

Table 1

Blood Glucose, Insulin, and Glucagon Levels of STZ-Diabetic Rats and Effects of Phlorizin Treatment and Glucose Administration[a]

	Glucose, mg/dL	Insulin, μU/mL	Glucagon, pg/mL
Control	141 ± 2	21.5 ± 2.0	82.7 ± 11.8
STZ-Diabetic	324 ± 34[b]	13.6 ± 1.8[b]	189.1 ± 36.6
STZ-DIAB-PHLO	102 ± 6[c]	8.1 ± 4.5[b,c]	286.0 ± 34.7[b]
STZ-DIAB-PHLO-GLUC	534 ± 24[b,c,d]	7.5 ± 3.0[b,c]	171.3 ± 20.0[d]

[a]Fasting blood glucose and insulin levels in control rats, STZ-diabetic rats, phlorizin-treated diabetic rats (STZ-DIAB-PHLO), and phlorizin-treated diabetic rats injected with glucose for 40 min (STZ-DIAB-PHLO-GLUC).

[b]$p < 0.05$ vs control group.

[c]$p < 0.05$ vs diabetic group.

[d]$p < 0.05$ vs diabetic-phlorizin group.

Table 2

Protein Yields and 5'-Nucleotidase Activity in Isolated Membrane Fractions from Rat Skeletal Muscle[a]

Fraction	Protein yield, μg/g of tissue			5'-Nucleotidase, nmol/min/mg protein		
	STZ-Diabetic			STZ-Diabetic		
Glycemia	DIAB High/chronic	PHLO Normal	GLUC High/acute	DIAB High/chronic	PHLO Normal	GLUC High/acute
CM	1884 ± 395	1805 ± 270	2142 ± 453	38.3 ± 8.9	42.2 ± 6.3	40.8 ± 4.4
PM	87 ± 24	77 ± 14	84 ± 18	187.9 ± 46.5	188.4 ± 47.9	194.9 ± 33.5

[a]Protein yield and 5'-nucleotidase activity are shown for crude unfractionated membranes (CM) and plasma membranes (PM) isolated from muscle of STZ-diabetic (DIA) rats, phlorizin-treated diabetic rats (PHLO), and phlorizin-treated diabetic rats injected with glucose for 40 min (GLUC). No significant differences in protein yields or 5'-nucleotidase were seen among the three groups of animals.

decreases insulin-stimulated glucose uptake in muscle. This glucose-induced insulin resistance may reflect the toxic effect of glucose *(12,13)*. Alternatively, we propose that it can represent a protective mechanism against excessive glucose uptake and accumulation *(6,7)*, perhaps sparing muscle cells from experiencing long-term diabetic complications *(14)*. This concept is supported by the finding of Zierath et al. *(15)* who showed that the impaired insulin-stimulated glucose transport of muscle strips from non-insulin-dependent diabetes mellitus (NIDDM) patients is normalized by incubating the muscles in normoglycemic conditions for 2 h. We have previously provided evidence that long-term hyperglycemia, independently from insulinemia, decreases the expression of the insulin-responsive GLUT4 transporter and its amount in the plasma membrane of diabetic rat skeletal muscle *(7)*. We thus suggested that the downregulating effect of hyperglycemia on plasma membrane GLUT4 protein amounts was responsible at least in part for glucose-induced insulin resistance in diabetic muscle *(7,14)*. Whether enhanced intrinsic activity of glucose transporters also occur in muscle cells of phlorizin-treated diabetic rats remains to be explored. In intact non-diabetic rats, long-term hyperglycemia was not found to reduce GLUT4 content in isolated muscle plasma membrane presumably, because hyperglycemia was accompanied by hyperinsulinemia and the effects of hyperinsulinemia and hyperglycemia can counteract each other with respect to translocation of GLUT4. Nevertheless, glucose-infused rats had a significant impairment in insulin mediated glucose uptake, suggesting that high glucose suppresses GLUT4 intrinsic activity in normal rats *(16)*. Also, in fat cells from pancreatectomized diabetic rats, restoration of glucose transport activity after normalization of blood glucose with phlorizin was related to an increased intrinsic activity of the transporter proteins, and not to the GLUT4 abundance in the plasma membrane *(17)*.

In addition to its chronic influence, glucose also appears to regulate acutely its own uptake in muscle cells in the absence of any change in insulinemia. Indeed, Richter et al. *(8)* and Hansen et al. *(9)* reported that perfusion of rat hindquarter muscles with high glucose concentrations caused an acute (as rapid as 30 min), concentration-dependent

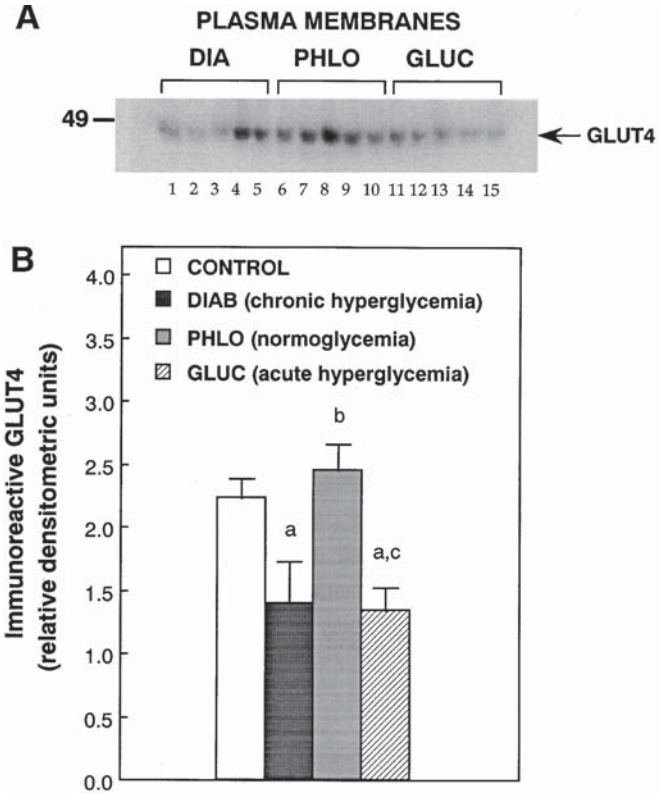

Fig. 1. **(A)** Representative Western blot of GLUT4 content in plasma membranes isolated from skeletal muscle of STZ-diabetic rats (DIAB, lanes 1–5), phlorizin-treated diabetic rats (PHLO, lanes 6–10), and phlorizin-treated diabetic rats injected with glucose for 40 min (GLUC, lanes 11–15). The migration of the 49-kDa mol-wt standard is shown on the left. GLUT4 migrated at 45–46 kDa. **(B)** Mean GLUT4 content in plasma membranes (PM) isolated from skeletal muscle of the same rats. Membrane fractions were isolated and analyzed by Western blotting as described in Materials and Methods. Data are means ± SE of 4–5 independent membrane preparations from 4–5 different animals in each group. A control sample was run on all gels in order to compare the relative densitometric units of GLUT4 immunoreactivity between samples ran on different gels. $^{a}p < 0.05$ vs control group. $^{b}p < 0.05$ vs diabetic (DIAB) group. $^{c}p < 0.05$ vs diabetic-phlorizin (PHLO) group.

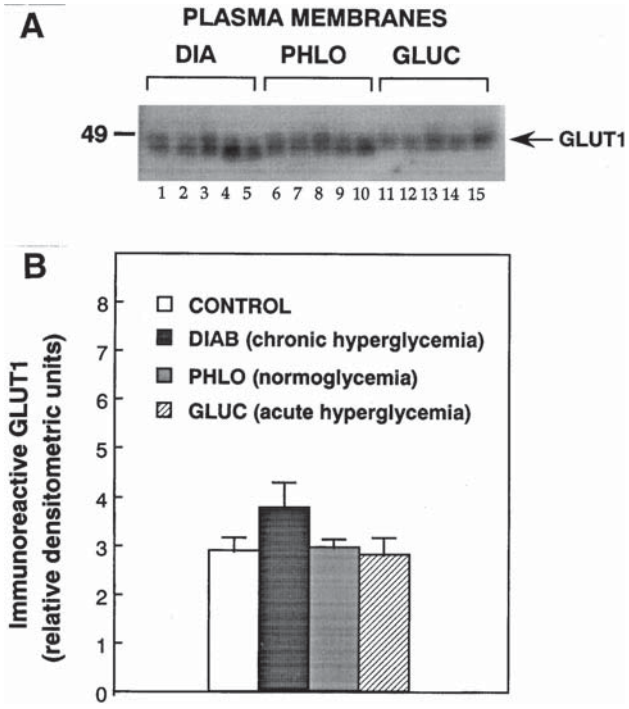

Fig. 2. **(A)** Representative Western blot of GLUT1 content in plasma membranes isolated from skeletal muscle of STZ-diabetic rats (DIAB, lanes 1–5), phlorizin-treated diabetic rats (PHLO, lanes 6–10), and phlorizin-treated diabetic rats injected with glucose for 40 min (GLUC, lanes 11–15). GLUT1 migrated at 46–47-kDa. **(B)** Mean GLUT1 content in plasma membranes (PM) isolated from skeletal muscle of the same rats. *See* legend to Fig. 1 for more details. Data are means ± SE of 4–5 independent membrane preparations from 4–5 different animals in each group. No statistically significant effects of the treatments were observed.

reduction in insulin-stimulated glucose transport. The results of the present study suggest that glucose rapidly modulates its transport in skeletal muscle by altering GLUT4 abundance in the cell surface, where glucose transport takes place. Indeed, an acute elevation of glycemia in phlorizin-treated diabetic rats was found to decrease plasma membrane GLUT4 transporter abundance back to values observed in untreated diabetic animals. This effect of a sudden rise in glycemia on plasma membrane GLUT4 amounts may provide a cellular basis for the previous observations of a rapid inhibition of insulin-stimulated glucose transport in the presence of high glucose levels. In our experimental model, the elevation in blood insulin levels that usually follows iv glucose administration was prevented by the use of diabetic animals. In that regard, it is interesting to note that residual β-cells lost the ability to respond to the sudden rise in glucose levels by changing insulin secretion. This is, however, essential for assessing the effect of hyperglycemia independently of insulin. Even a small increase in insulin in such experiments could lead to erroneous conclusions regarding the role of hyperglycemia.

Although an acute restoration of hyperglycemia reduced the plasma membrane GLUT4 content in muscle of phlorizin-treated STZ-diabetic rats, the GLUT4 abundance in the unfractionated crude membrane fraction was not changed. The failure to detect a reduction of GLUT4 in crude membranes is not surprising, since their recovery is 20- to 25-fold greater than that of plasma membranes (Table 2). Therefore, even a significant decrease in plasma membrane GLUT4 content would have a minimal impact on GLUT4 abundance in crude membranes. On the other hand, the lack of a significant reduction in total GLUT4 levels in muscle of glucose-infused rats may suggest that the transporter was redistributed to some intracellular compartment, the nature of which remains to be established.

In contrast to GLUT4, acute re-establishment of hyperglycemia in phlorizin-treated diabetic rats did not reduce GLUT1 protein levels in the plasma membrane. This is

consistent with the observation that the net uptake of glucose in muscle is increased by mass action in the presence of hyperglycemia. Indeed, it was reported that doubling the plasma glucose concentration while maintaining basal insulin levels almost doubled glucose utilization in skeletal muscle *(18)*. Increased glucose uptake in muscle by mass action is likely to be mediated mainly by the GLUT1 protein, since this high K_m transporter is believed to be far from saturated at normoglycemia *(19,20)*. In contrast, because GLUT4 is nearly saturated at normoglycemia *(19)*, it probably does not play a major role in an increased mass action of glucose.

In addition to its effect on plasma membrane GLUT4 levels, glucose may inhibit insulin-stimulated glucose transport by other mechanisms. Indeed, Hansen et al. *(9)* recently proposed that glucose-induced insulin resistance could be caused by changes in intracellular milieu, since subsequent measurements of glucose uptake in isolated vesicles from high-glucose-exposed muscles were not found to be reduced. However, these authors did not measure the contents of either GLUT1 or GLUT4 transporters in their isolated membrane fraction. Thus, in their study, changes in plasma membrane glucose transporter contents cannot be excluded, since reductions in the amount of the GLUT4 protein may have been masked by increased levels of the GLUT1 transporter and/or modification of the intrinsic activities of both transporters. More importantly, the acute effect of a glucose challenge was investigated in insulin-stimulated normal rat muscle in their study, whereas glucose was administered to diabetic rats in the basal state in our study. However, it is difficult to compare their study and ours because the amounts and activities of GLUT1 and particularly GLUT4 are affected both by hyperglycemia and insulin levels.

In summary, glucose acutely reduces plasma membrane GLUT4, but not GLUT1 protein content in diabetic rat skeletal muscle, independently from insulinemia. It is proposed that acute glucose-induced insulin resistance in rat skeletal muscle could be caused in part by a rapid decrease in plasma membrane GLUT4 transporters. The rapidity of the glucose effect on plasma membrane GLUT4 abundance further suggests that it could represent a regulatory rather than toxic response to ambient glycemia.

Materials and Methods

Animals

The procedure used was approved by the local ethics committee. Male Sprague-Dawley rats (Charles River, Montreal, Quebec, Canada) were rendered diabetic by the administration of a low dose of STZ (65 mg/kg) dissolved in saline (day 0). In this particular model, fasting insulinemia was only moderately reduced, although being very low relative to the prevailing hyperglycemia. Other rats where injected with saline only and used as controls. Glycemia was normalized in some STZ-diabetic animals by phlorizin treatment from days 3–6 as detailed previously *(7)*. Rats from all groups were fasted overnight before the day of the experiment (d 7). Half of the phlorizin-treated STZ-diabetic rats received intermittent bolus injections of glucose over a 40-min period to raise blood glucose acutely. Glucose was injected in conscious rats through a catheter fitted in the jugular vein. The catheter was implanted under pentobarbital anesthesia (1.0 mL/kg) 3 d before the experimental day. Plasma glucose levels were monitored every 5 min to ensure that glycemia was maintained at >400 mg/dL during the infusion. Animals from all groups were then sacrificed by decapitation, and hindlimb muscles were rapidly excised, frozen in liquid nitrogen, and kept at –80°C until processed for membrane isolation.

Isolation of Plasma Membranes

Plasma membranes were isolated by differential centrifugation and sucrose density gradient fractionation as previously described in ref. *(21)* and modified in ref. *(22)* using 10–15 g of hindlimb muscles of mixed-fiber composition. This fractionation procedure has been previously validated and the membranes have been amply characterized *(23–25)*. Briefly, muscle homogenates are subjected to differential centrifugations to yield crude membranes. These are further fractionated by sucrose gradient centrifugation. Membranes banding atop 25% sucrose are enriched with plasma membranes.

Western Blot Analysis

Membranes (20 μg of protein) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels, electrophoretically transferred to polyvinylidene difluoride (PVDF) filter membranes for 2 h, and immunoblotted exactly as previously described *(22)*. The amount of proteins loaded on the gel was verified by staining the transferred proteins on the PVDF membranes with either Ponceau S or India ink. PVDF membranes were incubated for 1 h at room temperature with Tris-saline (50 m*M* Tris-HCl, pH 7.4, 150 m*M* NaCl) containing 0.04% of the nonionic surfactant NP 40 and 3% bovine serum albumin (BSA) (buffer A), followed by overnight incubation at 4°C with either polyclonal GLUT4-specific antibody (IRGT, 1:500 dilution) or the polyclonal GLUT1-specific antibody (RaGLUTRANS, 1:1000 dilution) in buffer A (both antibodies obtained from East Acres Biologicals, Southbridge, MA). The PVDF membranes were then washed three times in buffer A (without BSA) followed by a 1-h incubation with ^{125}I-labeled protein A (2 μCi/10 mL) in buffer A, washed three times in buffer A (without BSA), air-dried, and exposed to XAR-5 Kodak film for 12–48 h. Autoradiographs were quantitated by laser scanning densitometry using a LKB Bromma ultrascan XL enhancer laser scanner with on-line analysis by a Packard Bell computer. Autoradiograms were exposed only enough to provide bands in the linear range of densi-

tometry for quantitation. A control membrane sample (crude unfractionated membranes) was run on every gel and used for comparing samples from different gels.

Statistics

Data were statistically analyzed by one-way analysis of variance. Differences between experimental groups were located with the PLSD *post hoc* comparison test which was applied when the F ratio was significant ($p < 0.05$).

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