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Effects of β-Naphthoflavone on Insulin Receptor Binding and Protein Kinase Activity in Rat Liver and Placenta

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SUMMARY

Studies investigating the effects of β-naphthoflavone (βNF) on insulin receptor binding and its intrinsic protein kinase activity in rat liver and placenta were performed. Membranes were prepared from maternal liver and placenta on gestation day 11 and used for [^{125}I]insulin radioreceptor assay. Scatchard analysis showed that association constants (K_a) for high affinity binding sites were similar for placental and liver membrane. The administration of βNF, 15 mg/kg, 1 day before study did not alter the specific binding of insulin to liver membranes, whereas ligand binding to placental preparations was decreased 40% from control. Scatchard analysis of binding to placental membranes suggests that βNF treatment was associated with a change in the number of high affinity binding sites. In further studies membrane receptors were solubilized and partially purified by wheat germ agglutinin affinity chromatography for protein kinase assay. Insulin stimulated the phosphorylation of the M_r 95,000 subunit of the receptor in lectin-purified membrane proteins from liver and placenta. In liver receptor preparations, βNF treatment was associated with a nearly 3-fold increase in the insulin-stimulated phosphorylation of the 95-kD protein. In contrast, placental receptor preparations showed a 40% decrease in the extent of autophosphorylation following βNF treatment. Insulin-stimulated phosphorylation of an exogenous substrate poly(Glu$_4$,Tyr) also showed a divergent pattern of changes in liver and placental receptors following βNF treatment. In studies during late gestation (day 18), βNF treatment was also associated with an increase in liver receptor kinase activity, whereas placental receptors showed a decrease in autophosphorylation. Thus, acute treatment with βNF during mid and late gestation was associated with significant alterations in insulin receptor protein kinase activity, and data suggest that fetal insulin receptors may respond in a different manner than maternal receptors to polyaromatic compounds like βNF. The observed effects of βNF on liver and placental receptor kinase activity may be related to alterations in insulin function in the regulation of pregnancy and fetoplacental growth.

Exposure to polyaromatic compounds during pregnancy has been associated with low birth weight in rodents (1, 2), monkeys (3), and humans (4). Late fetoplacental growth was selectively impaired after the administration of βNF and 3-methylcholanthrene to pregnant rats during mid-gestation (2). Thus, data suggest that polyaromatic compounds interfere with major physiologic regulators of fetoplacental growth. In this regard, insulin has been shown to be a significant fetal growth factor during late gestation (5, 6). Human placenta is a rich source of insulin receptor which has been extensively characterized for its physicochemical and protein kinase properties (7). Our recent studies have characterized the ontogeny of insulin receptors in the rat hemochorial placenta (8). Insulin receptors in rat placenta and maternal liver are found to be similar in subunit molecular weight, whereas functional differences exist in insulin-stimulated protein kinase activities. The present study further investigated whether βNF administration to pregnant rats was associated with biochemical alterations in placental insulin receptors.

Recent evidence supports an interaction of polyaromatic compounds with growth factor receptors. The binding of EGF to cell surface receptors was reduced by polyaromatic compounds in association with the Ah receptor in several cell lines (9–11). TCDD administered *in vivo* decreased EGF binding to liver plasma membranes, increased membrane protein kinase activities, and elicited symptoms of excess EGF in newborn mice (12, 13). In human pregnancy, exposure to halogenated aromatic compounds and cigarette smoke has been associated with a decrease in EGF receptor protein kinase activity in the placenta (14, 15). At present, however, little is known about the interaction of polyaromatic compounds with other growth factor receptors in development. In view of the reported adverse

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ABBREVIATIONS: βNF- β-naphthoflavone (5,6-benzoflavone); EGF, epidermal growth factor; Ah, aryl hydrocarbon; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; poly(Glu$_4$,Tyr), copolymer of glutamate-tyrosine, 4:1; PMSF, phenylmethylsulfonyl fluoride; WGA, wheat germ agglutinin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

effects of βNF on rat fetoplacental growth, the present study examined the interaction of this polyaromatic compound with placental insulin receptor.

Materials and Methods

Chemicals. Bovine serum albumin, fraction V, was purchased from U.S. Biochemical Co. (Cleveland, OH). Porcine insulin, PMSF, adenosine 5′-triphosphate, cytidine 5′-triphosphate, poly-(Glu_4,Tyr), *N*-acetyl-D-glucosamine, SDS-PAGE protein standard kit, and βNF were from Sigma Chemical Co. (St. Louis, MO). βNF was recrystallized from ethanol. Chemicals for SDS-PAGE were purchased from Bio-Rad (Hempstead, NY). Agarose-bound wheat germ agglutinin was obtained from Vector Laboratories, Inc. (Burlingame, CA). ^{125}I-Human insulin labeled at tyrosine B-26 (specific activity 2000 Ci/mmol) and [γ-^{32}P]ATP (specific activity 3000 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL).

Animals. Pregnant rats were purchased from Holtzman Co. (Madison, WI) and housed in wire cages on a 12-hr light cycle. Day 0 of gestation was designated as the day on which sperm was detected in the vaginal smear. βNF was administered (15 mg/kg, intraperitoneally) in corn oil to pregnant rats on gestation day 10 or 17. Animals were anesthetized with sodium pentobarbital on days 11 or 18 prior to laparatomy. Hemochorial placentae (basal zone and labyrinth) were pooled from a single litter on day of 18 gestation, whereas placental tissue from two or more litters was pooled from day 11 animals.

[^{125}I]Insulin Binding. Microsomal membranes of placental tissue and maternal liver were prepared by differential centrifugation as described by Pilistine *et al.* (16). During the membrane preparations, PMSF, aprotinin, and leupeptin were added to the buffer as protease inhibitors. Protein concentration of microsomal membrane was measured by the method of Lowry *et al.* (17) with bovine serum albumin as a standard.

Microsomal membranes (250 μg of protein in 50 μl) were incubated in triplicate with [^{125}I]insulin (40,000 cpm, 0.05 nM) and 0.1 M sodium phosphate buffer, pH 7.5, containing 1.0% bovine serum albumin (w/v) (buffer A) in a final reaction volume of 200 μl for 30 min at 25° for the measurement of total binding. Nonspecific binding was determined in the presence of 2.5 μM unlabeled porcine insulin. The reaction was terminated by addition of 0.5 ml of ice-cold buffer A. Membrane-bound [^{125}I]insulin was separated immediately by centrifugation at 10,000 × *g* for 3 min in an Eppendorf microcentrifuge followed by aspiration of the supernatant; the pellet was washed with another 0.5 ml of ice-cold buffer A, recentrifuged for 1 min, and the supernatant was aspirated. The resultant pellets were counted in a Beckman Gamma 5500 counter. Specific binding was obtained by subtracting the radioactivity of nonspecific binding from total binding and expressed as a ratio of the specific binding versus unbound [^{125}I]insulin (bound/free or *B*/*F*).

[^{125}I]Insulin degradation was tested frequently during the course of experiments by a trichloroacetate precipitation method (18). The extent of degradation was used to correct the total radioactivity added in the reaction mixture. To determine receptor association constant (K_a) and binding sites (B_{max}), Scatchard analysis (19) was performed as described in the standard assay except that 0–10 μM unlabeled insulin was added to the incubation mixture. Data from Scatchard plots were analyzed by the LIGAND computer program based on a two-site model as described previously (8).

WGA chromatography. Insulin receptors were purified from placenta and liver membranes by WGA chromatography (20, 21). Microsomal membranes were solubilized in 50 mM HEPES, 10 mM $MgSO_4$, 1 mM PMSF, and 1% Triton X-100, pH 7.6. The solubilized proteins (20–30 mg) were recycled three times over a column containing 3.5 ml of agarose-bound WGA at 4°. The column was washed with 40 ml of washing buffer (50 mM HEPES, 150 mM NaCl, and 0.1% Triton X-100, pH 7.6), and the receptors were eluted from the column with 10 ml of the same buffer containing 0.3 M *N*-acetyl-D-glucosamine and collected at 2.0 ml/fraction. The eluates were monitored for protein concentration by UV spectrometry at 280 nm. Three eluates with highest absorbance at 280 nm were combined and protein concentration was determined using the Bio-Rad microassay (Manual of Bio-Rad Protein Kit). Insulin receptor in WGA-purified preparation was assayed by the method of Hedo *et al.* (20).

Insulin receptor phosphorylation. Insulin receptor phosphorylation was performed by a modification of the method of Lowe *et al.* (21). Lectin-purified receptor preparations [containing a constant amount of insulin binding, 1% (*B*/*F*)] were preincubated in the absence or presence of 1.0 μM porcine insulin in a final volume of 80 μl of 50 mM HEPES buffer, pH 7.6, with 10 mM $MgSO_4$ and 1 mM PMSF. After 30 min at 22°, phosphorylation was initiated by the addition of 20 μl of reaction mixture to give final concentrations of 5 μM [^{32}P]ATP (specific activity 20 μCi/nmol), 1 mM cytidine-5-triphosphate, 3 mM $MnCl_2$, 20 mM $MgCl_2$, and 1 mM sodium vanadate ([^{32}P]ATP-mixture). After 10 min at 22°, the reaction was terminated by the addition of an equal volume of stopping solution (0.17 M Tris-HCl, 10% SDS, and 100 mM dithiothreitol, pH 6.8) and heating at 100° for 10 min. Aliquots (120 μl) were analyzed by SDS-PAGE followed by autoradiography. The intensity of ^{32}P-labeled protein bands shown on X-ray film was quantitated by Soft-Laser densitometer, model SF-TRFF (Biomed Instrument, Inc., Fullerton, CA).

Artificial substrate phosphorylation. The experiments were performed using a modified method of Rees-Jones *et al.* (22). Lectin-purified receptor preparations (12 μg) were preincubated in the absence or presence of 1.0 μM porcine insulin in a final volume of 100 μl of 50 mM HEPES buffer, pH 7.6, with 10 mM $MgSO_4$ and 1 mM PMSF and 0.2 mg of poly(Glu_4,Tyr). After 30 min at 22°, phosphorylation was initiated by the addition of 20 μl of [γ-^{32}P]ATP-mixture. After 10 min at 22°, the reaction was terminated by spotting 35 μl of the reaction mixture on 3 MM Whatman filter paper; samples were run in triplicate. The filter paper was then extensively washed with 10% trichloroacetic acid containing 10 mM sodium pyrophosphate, followed with 95% ethanol and ether. The filter paper was dried and counted. The counts of ^{32}P incorporated in the absence of porcine insulin was subtracted from counts in the presence of porcine insulin to obtain the specific phosphorylation activity. One unit of kinase activity was defined as the incorporation of 1 fmol of phosphate into 1 μg of poly(Glu_4,Tyr).

Results

Insulin binding. The specific binding of [^{125}I]insulin to membranes prepared from maternal liver and placenta increased linearly with protein concentrations of 0.2–1 mg over a 30-min time period. While initial experiments established that placental membranes degraded insulin much more extensively than liver membranes, it was further determined that insulin labeled with ^{125}I at tyrosine B_{26} was more resistant than tyrosine A_{14} to degradation. For this reason the standard binding assay used [^{125}I]insulin labeled at tyrosine B_{26} and the extent of ligand degradation was determined in each experiment. The specificity of insulin binding to rat liver membranes has been well characterized (23). In placental membranes, unlabeled porcine and chicken insulin showed typical displacement curves (IC_{50} = 65 nM) for competition with [^{125}I]insulin binding, whereas insulin-like growth factor I, human growth hormone, glucagon, relaxin, and desoctapeptide insulin had no inhibitory effect on tracer binding (8). Scatchard analysis of specific binding to membranes from maternal liver and placenta demonstrated curvilinear plots as shown in Fig. 1. Scatchard plots analyzed by the LIGAND computer program indicate that association constants (K_a) for high affinity binding sites were similar for day 11 placental and liver membranes, whereas the number of binding sites was substantially greater for liver.

Data in Table 1 show the effects of a 1-day exposure to βNF

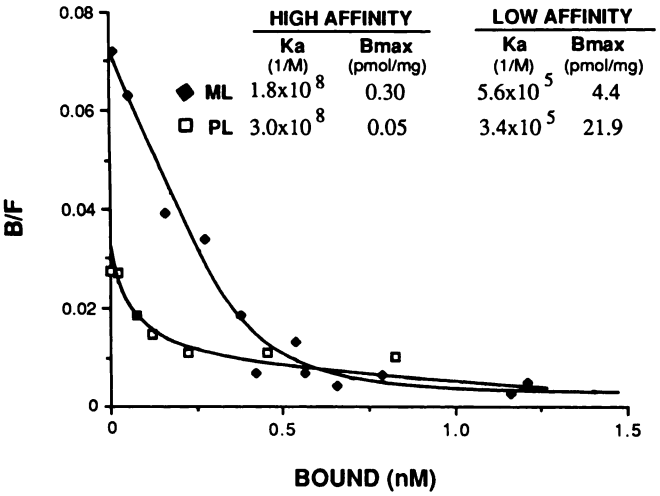

Fig. 1. Scatchard plots of [^{125}I]insulin binding to membranes prepared from rat placenta (*PL*) and maternal liver (*ML*). *B/F* ratio of the hormone is plotted as a function of insulin bound to membrane preparations. Data are expressed as the mean of triplicate determinations.

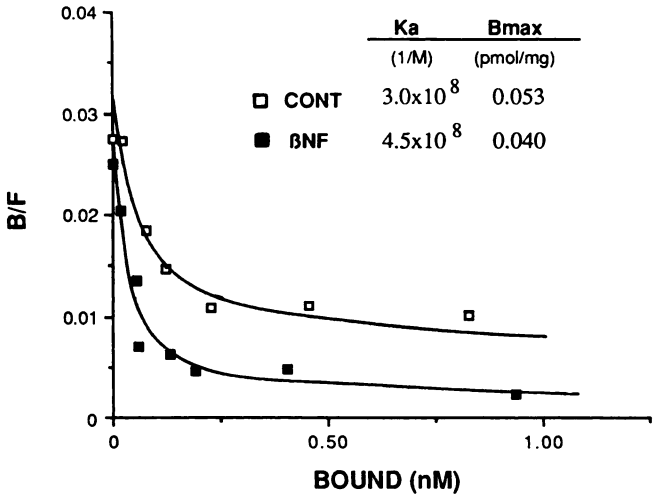

Fig. 2. Scatchard plots of [^{125}I]insulin binding to placental membranes from control (*CONT*) and βNF-treated rats. *B/F* ratio of the hormone is plotted as a function of insulin bound to membrane preparations. Data are expressed as the mean of triplicate determinations.

TABLE 1

Effects of βNF on [^{125}I]insulin binding to placenta and liver membranes

Total binding was measured with [^{125}I]insulin (0.05 nM) incubated in the presence of microsomal membranes (250 μg of protein) for 30 min as described under Materials and Methods. Nonspecific binding was determined in the presence of 2.5 μM unlabeled porcine insulin. Data are expressed as the ratio of specific binding versus unbound [^{125}I]insulin (% *B/F*). All data are corrected for the extent of [^{125}I] insulin degradation during the incubation. Values are the means ± standard error of five to six animals (shown in parentheses), each performed in triplicate.

	Day 11 gestation
	% *B/F*
Maternal liver	
Control	11.9 ± 0.96 (6)
βNF	13.9 ± 0.81 (6)
Placenta	
Control	1.75 ± 0.09 (5)
βNF	1.01 ± 0.06* (5)

*$p < 0.001$.

on insulin binding to membranes prepared from liver and placenta on gestation day 11. Specific binding of [^{125}]insulin per 250 μg of liver membrane protein was not significantly altered by βNF treatment. In contrast, specific binding to 250 μg of placental membrane protein was decreased significantly by 40% following βNF treatment. Scatchard analysis of binding to placental membranes (Fig. 2) suggests that βNF treatment was associated with a change in the number of high affinity binding sites.

Insulin receptor phosphorylation. In subsequent experiments, the intrinsic protein kinase activity of insulin receptors was examined. Membranes from liver and placental tissue were solubilized in 1% Triton X-100 and soluble glycoproteins were partially purified by WGA affinity chromatography. In soluble, lectin-purified receptor preparations from placenta, insulin binding was purified an average of 30-fold with 50% recovery. Receptor preparations from maternal liver showed an average 5-fold purification of insulin binding with 30% recovery from crude membranes. βNF treatment was not associated with any apparent change in the purification and percentage recovery of insulin binding during solubilization and lectin affinity chromatography procedures.

Insulin stimulated the phosphorylation of an M_r 95,000 protein in purified receptors from liver and placenta, which is consistent with the β-subunit of the insulin receptor (Figs. 3 and 4). For kinase assays, the tracer binding capacity was adjusted by dilution to a constant 1% *B/F* in each of the receptor preparations from the liver and placenta of control and βNF-treated animals. The same amount of insulin binding was applied to each lane of the gels in Figs. 3 and 4. On gestation day 11, βNF treatment was associated with a marked increase in the insulin-stimulated phosphorylation of the 95-kDa protein in liver receptor preparations. Densitometric scan of the autoradiogram in Fig. 3 showed that the extent of ^{32}P incorporation into the 95-kDa protein in βNF preparations was increased 2.8-fold from control. βNF was also associated with the increased phosphorylation of a 170kDa hepatic protein which was independent of insulin stimulation. In contrast, placental receptor preparations from gestation day 11 showed a 40% decrease in the extent of autophosphorylation following βNF treatment (Fig. 4). Thus, data indicate that an acute exposure to βNF during midgestation was associated with increased autophosphorylation of liver insulin receptors, while placental receptors showed a decrease in insulin stimulated kinase activity.

Late gestation receptors. The differential effect of βNF administration on autophosphorylation activity of liver and placental receptors on gestation day 11 led us to further examine receptors during late gestation. We have previously reported that both maternal liver and placenta show a decrease in insulin binding from mid- to late gestation (8). In the present study, pregnant rats were treated with βNF on gestation day 17 and tissues were studied on day 18. Specific binding of [^{125}I] insulin to 250 μg of liver membrane protein was 6.9 ± 0.7% *B/F* and 4.5 ± 0.6% for control and βNF, respectively. For placental membranes from gestation day 18, specific binding of insulin was 1.17 ± 0.07% *B/F* and 0.7 ± 0.06% ($p < 0.001$) for control and βNF, respectively. Thus, βNF treatment during late gestation was not associated with any marked change in insulin binding to liver membranes, whereas binding to placental membranes was decreased significantly, 40% from control.

Membrane glycoproteins were solubilized and purified by lectin chromatography from liver and placental membranes on

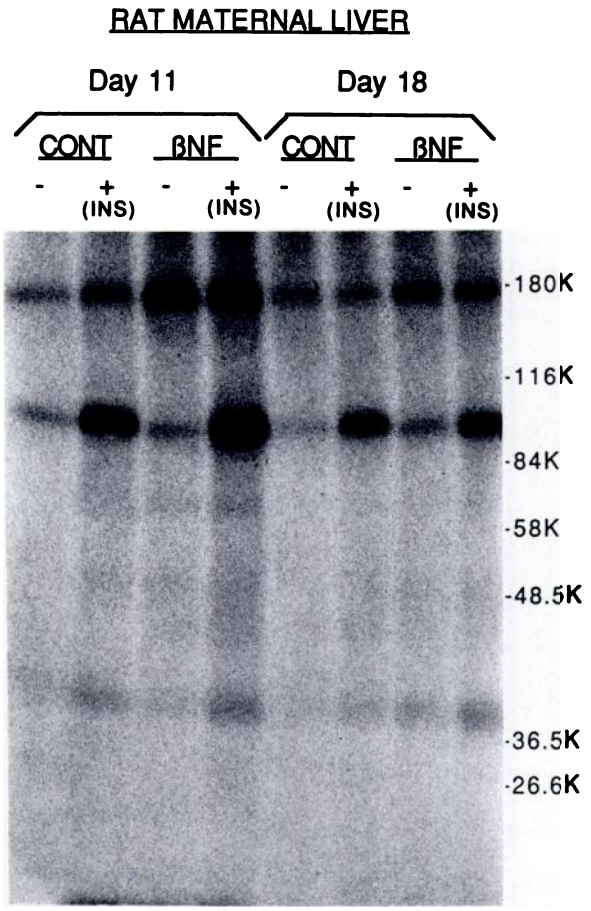

Fig. 3. Autoradiograms of SDS-polyarcylamide gels show insulin-stimulated phosphorylation of proteins in solubilized, lectin-purified preparations of maternal liver membranes. The amount of protein added to the reaction mixture (4–10 μg) was adjusted to obtain 1% *B/F*. Phosphorylation assays were conducted in the absence (−) or presence (+) of 1.0 μM insulin (*INS*). Liver membranes were prepared on days 11 and 18 of gestation from control and βNF-pretreated animals.

day 18 and further examined for protein kinase activity. All receptor preparations were diluted so that kinase assays were conducted in the presence of a constant 1% *B/F* in order to normalize for gestational and βNF-related differences in binding. Data in Fig. 3 for day 18 liver receptor preparations show that βNF treatment was again associated with an increase in insulin-stimulated phosphorylation of the 95-kDa protein. Densitometric scans of autoradiograms indicate that the extent of ^{32}P incorporation into hepatic receptor was increased 2.8-fold on day 11 and 1.3-fold on day 18 following βNF treatment. In contrast, study of placental receptor preparations from day 18 gestation showed that βNF was associated with a 40% decrease in the extent of autophosphorylation (Fig. 4). Thus, during both mid- and late gestation, βNF was associated with a decrease in autophosphorylation of the placental insulin receptor, while liver receptor preparations have increased kinase activity.

Exogenous substrate phosphorylation. In the autophosphorylation reaction, the insulin receptor serves as both enzyme and substrate. To measure insulin receptor kinase separate from its properties as substrate, we assayed the insulin-stimulated phosphorylation of an exogenous substrate, poly (Glu_4,Tyr). Data in Table 2 on tyrosine kinase activity toward poly(Glu_4,Tyr) were normalized for constant *B/F* and expressed as insulin-stimulated kinase/(*B/F*). Purified receptor preparations from maternal liver exhibited higher insulin-stimulated tyrosine kinase activity than did placental receptor preparations. Following βNF treatment, liver receptors from mid- and late gestation showed 2.1- and 1.5-fold increases in insulin-stimulated phosphorylation, respectively. For the placenta, however, βNF treatment was associated with 15–35% decreased tyrosine kinase activity in receptors purified from mid- and late gestation placentae. Thus, the differential effects of βNF on kinase activity of placenta and liver insulin receptor were comparable for both autophosphorylation and the exogenous substrate.

Discussion

The insulin receptor of rat liver has been well characterized for its physicochemical and protein kinase properties (24, 25). Insulin stimulates the phosphorylation of its 95-kDa β-subunit, as well as tyrosine residues in other membrane proteins, exogenous proteins, and synthetic peptides (26). Data from several laboratories suggest that insulin receptor phosphorylations are involved in autoregulation and in the intracellular transmission of the insulin signal (22, 25–27). This laboratory has recently described the ontogeny of insulin receptors in the rat hemochorial placenta during gestation (8). The insulin receptor α- and β-subunits in rat liver and placenta were found to have similar molecular weights, and Scatchard analysis further demonstrated that dissociation constants (K_d) were similar for both liver and placenta preparations. A comparison of mid- and late gestation placentae in our previous study (8) showed that protein kinase activity was far more extensive for receptor preparations from day 11 placenta, data which suggest that α- and β-subunits are coupled and more functional during mid-gestation. The observation that specific binding of [^{125}I]insulin to maternal liver membranes decreased from gestation day 11 to day 18 further suggests that pregnancy-related factors, probably hormonal, play a role in regulation of the hepatic insulin receptor.

In the present study, the acute administration of βNF was associated with significantly decreased binding of [^{125}I]insulin to membranes prepared from placenta on gestation days 11 and 18, in the absence of any comparable effect on liver membrane binding. Alterations in hepatic binding of insulin and EGF have been reported following acute exposure to the hepatocarcinogens 2-acetylaminofluorene (28, 29) and diethylnitrosamine (30) which reflected an apparent decrease in the number of receptors. The present study is the first report of an alteration in placental insulin binding associated with exposure to polyaromatic compounds. Although alterations in liver insulin receptors have been associated with chronic exposure to the tumor promoters TCDD (31) and phenobarbital (32), it is difficult to distinguish direct chemical-mediated events from changes due to long-term toxicity and altered growth state. In this regard, the present study used a 1-day treatment protocol to avoid long-term problems with overt toxicity. In several cell lines, benzo(*a*)pyrene and other inducers of cytochrome P_1-450 have been reported to show acute inhibition of EGF receptor binding which was mediated by the Ah receptor (9–11). The dose of βNF administered in the present study clearly induced Ah hydroxylase activity in maternal liver and placenta (33), and experiments are in progress to investigate the relationship between Ah hydroxylase induction and decreased insulin binding in the rat placenta.

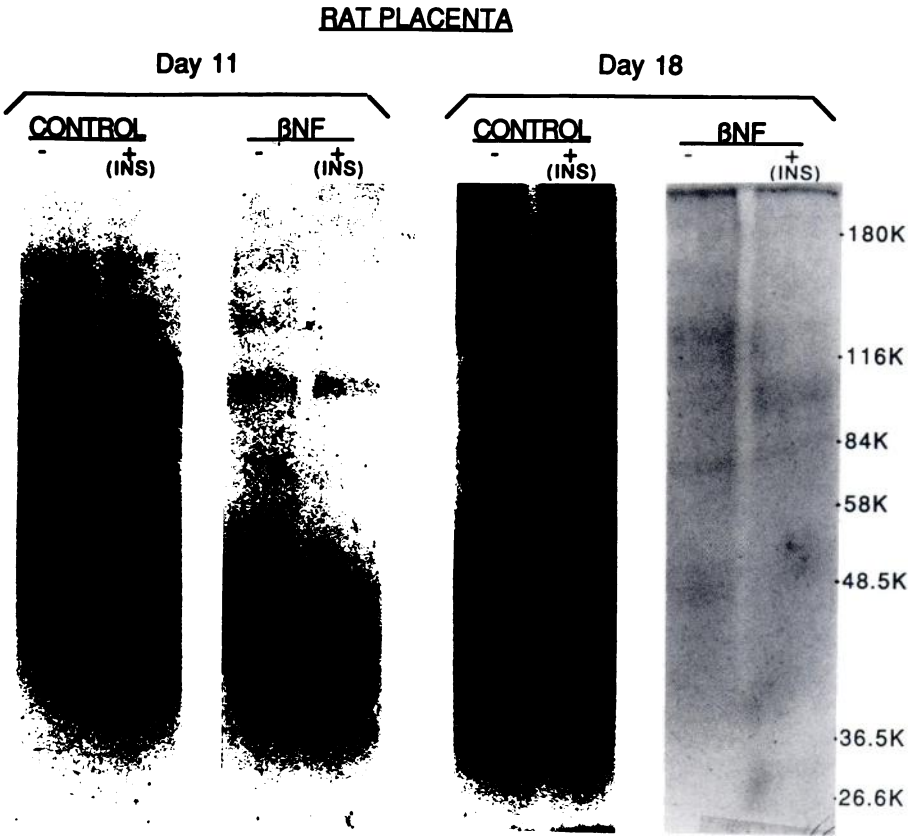

Fig. 4. Insulin-stimulated phosphorylation of proteins in solubilized, lectin-purified preparations of placental membranes. Assay conditions and samples are as described in Fig. 3. Reaction mixtures contained 6 μg of protein for day 11 preparations, while 16–24 μg of protein were used for day 18 samples.

TABLE 2

Effects of βNF on tyrosine kinase activity of insulin receptors

The kinase activity of solubilized, lectin-purified membrane proteins was assayed in the presence of poly(Glu_4, Tyr) substrate. The incorporation of ^{32}P was measured as that stimulated by 1 μM insulin in the presence of 3.5 μg of purified proteins during a 10-min incubation. Values are the means ± standard errors of two separate experiments each conducted in triplicate samples.

	Kinase activity		
	−Insulin	+Insulin	Δ Kinase activity
	fmol/μg Glu, Tyr/(B/F)		
Maternal liver			
Day 11			
Control	40 ± 13	116 ± 28	76 ± 15
βNF	87 ± 24	248 ± 61	162 ± 38
Day 18			
Control	59 ± 23	129 ± 31	70 ± 8
βNF	68 ± 16	172 ± 20	105 ± 4
Placenta			
Day 11			
Control	44 ± 18	89 ± 15	45 ± 3
βNF	42 ± 15	81 ± 2	39 ± 13
Day 18			
Control	124 ± 24	163 ± 29	40 ± 6
βNF	80 ± 22	108 ± 10	29 ± 13

The presence of intrinsic tyrosine kinase in receptors for insulin, EGF, and other polypeptide growth factors has been proposed to represent a general mechanism in transmembrane signaling (26, 27). In the present study, solubilized, lectin-purified insulin receptors were used to investigate functional alterations associated with βNF treatment during pregnancy. Although insulin binding to maternal liver membranes was not altered by βNF treatment, purified receptor preparations showed a marked increase in protein tyrosine kinase activity. In contrast, our studies of placental insulin receptor found that βNF administration was associated with significantly decreased ligand binding, decreased autophosphorylation, and reduced tyrosine kinase activity. The divergent changes in insulin receptor kinase activity for maternal liver and placenta suggest that fetal insulin receptors may respond in a different manner to polyaromatic compounds like βNF. Other laboratories have reported that exposure to the hepatocarcinogen diethylnitrosamine resulted in an acute, dose-dependent decrease in ligand binding and autophosphorylation for both insulin and EGF receptors in liver membranes (30). A recent study of women exposed to polychlorinated biphenyls in contaminated rice oil found that placental tissue showed a dose-dependent decrease in EGF binding and receptor autophosphorylation, and this change was correlated significantly with a decrease in birth weight (14). Our studies with placentas from women who smoke cigarettes have found that protein kinase activity of EGF receptors was markedly decreased, whereas insulin receptor kinase activity was normal (15). These data provide further evidence that biochemical alterations in growth factor receptor kinase activity may be a mechanism by which environmental chemicals interfere with human placental and fetal development.

Although the mechanisms which underlie these xenobiotic-related changes are not understood, there is substantial evidence to support a role for insulin receptor kinase in insulin action. An association of insulin resistance with impaired insulin receptor kinase activity has been reported in diabetes (34, 35) and obesity (36, 37), as well as in cultured rat hepatoma cells (38). Conversely, insulin-stimulated receptor autophosphorylation was enhanced by starvation and insulin treatment (39). The activation of insulin receptor kinase activity in rat

adipocytes correlated significantly with an increase in insulin-like growth factor II binding and receptor translocation (40). Recent evidence that several key regulatory enzymes in glycolysis act as substrates for the insulin receptor kinase *in vitro* (41) provides further support for the hypothesis that insulin receptor activation plays a role in mediating the response to insulin. Finally, the reported stimulation of autophosphorylation of purified insulin receptors *in vitro* by calmodulin (42), *src* kinase (43), and phosphatidylinositol (44) further suggests a role for other transmembrane signaling systems in the regulation of insulin receptor function.

In summary, the present study presents evidence that insulin receptor function is significantly altered during pregnancy by exposure to the polyaromatic compound βNF. Experiments are in progress to characterize biochemical mechanisms which underlie receptor alterations and to elucidate whether there is a relationship to induction of Ah hydroxylase activity in rat liver and placenta. Finally, our long-range goal is to determine whether the intrauterine growth retardation observed with βNF is mediated by altered insulin action on fetal and placental tissues.

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