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Interaction of Bovine Growth Hormone with Buffalo Adipose Tissue and Identification of Signaling Molecules in Its Action

R. Sodhi and Y. S. Rajput[1,] *

Animal Biochemistry Division, National Dairy Research Institute, Karnal-132001, India

ABSTRACT : Results on localization of growth hormone receptor (GHR), interaction of growth hormone (GH) with receptor in buffalo adipose tissue and identification of activated signaling molecules in the action of GH are presented. Bovine GH (bGH) was labeled with fluorescein or biotin. Fluorescein-labelled bGH was used for localization of GHRs in buffalo adipocytes. The receptors were present on the cell surface. The affinity of binding of GH to its receptor was determined by designing an experiment in which buffalo adipose tissue explants, biotinylated GH and streptavidin-peroxidase conjugate were employed. The affinity constant was calculated to be 2×10^8 M^{-1}. The receptor density on adipose tissue was found to be 1 femto mole per mg of tissue. Signalling molecules generated in the action of GH were tentatively identified by employing Western blot and enhanced chemiluminescence techniques using anti-phosphotyrosine antibody. Based on molecular weights of proteins reactive to anti-phosphotyrosine antibody, three signaling molecules viz. insulin receptor substrate, Janus activated kinase (Jak) and mitogen activated protein were tentatively identified. These signaling molecules appeared in a time (incubation time of explants with growth hormone) dependent way. The activation of Jak2 was confirmed by employing anti-Jak2 antibody in a Western blot. The activation of Jak2 occurred during 5 min incubation of buffalo adipose tissue explants with GH and incubation for an additional period, viz. 30 min. or 60 min., resulted in a drastic reduction in activation. The results suggest that Jak2 activation is an early event in the action of GH in buffalo adipose tissue. (**Key Words :** Growth Hormone, Growth Hormone Receptor, Signalling Molecules, Buffalo, Jak2)

INTRODUCTION

Growth hormone, also referred to as somatotropin, is a protein hormone produced in specific cells (somatotrophs) of the pituitary gland (Wallis, 1988). In terms of weight, it is the most abundant hormone of the anterior pituitary accounting for about 10% of dry weight. It is comprised of about 191 amino acids with a molecular weight of 22 kDa (Havel et al., 1989). The hormone has an antiparallel four helix bundle core with a characteristic 'up-up-down-down' topology (Abdel-Meguid et al., 1987). There are two binding sites for the growth hormone receptor on each molecule of growth hormone. There are at least three conformational epitopes in bovine growth hormone (Kumar and Rajput, 1999). Growth hormone is a growth promoting (Chen, 2001; Biswas et al., 2003) and metabolic hormone (Isaksson et al., 1985; Maksiri et al., 2006). The range of biological effects of growth hormone on growth and lactation is extraordinary. Administration of growth hormone can enhance milk yield by between 6 and 30% (Bauman et al., 1988; McBride et al., 1988; Burton and McBride, 1989; Chillard, 1989; Ludri et al., 1989; Prosser and Mepham, 1989).

Growth hormone produced in the pituitary is carried in the blood to target tissues. The receptors for growth hormone are present in liver (Herington et al., 1976; Donner, 1980), adipose tissue (Fagin et al., 1980; Gavin et al., 1982), lymphocytes, fibroblasts, macrophages, chondrocytes, β-islet cells and osteoblasts (Waters et al., 1989). The wide distribution of receptors in tissues has created interest in new roles of growth hormone and its mechanism of action. One molecule of growth hormone binds with two molecules of growth hormone receptor leading to dimerization of receptor, an essential step for expression of biological effects associated with growth hormone (Cunningham et al., 1991; Waters et al., 2006). Such interaction leads to activation of signaling molecules in a sequential way and controls expression of genes (Sodhi and Rajput, 2001; Woelfe et al., 2003a: Woelfe et al., 2003b: Brown et al., 2005; Rowland et al., 2005).

* Corresponding Author: Y. S. Rajput. Tel: +91-184-2259127, Fax: +91-184-2250042, E-mail: ys_rajput@rediffmail.com
[1] Dairy Chemistry Division.
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Compared to other species, adipose tissue from ruminants is richer in triglyceride content and isolated adipocytes aggregate quickly during isolation of single cell preparations (Fain, 1975). This also holds true for buffalo adipose tissue (Sodhi and Rajput, unpublished). The interaction of growth hormone with its receptor and identification of signaling molecules in its action are being studied in order to understand the mechanism of action of growth hormone. In the present work, the results on action of growth hormone in buffalo adipose tissue have been presented.

MATERIALS AND METHODS

Preparation of fluorescein labelled growth hormone

Fluorecein-labelled growth hormone was prepared by the method of Eshet et al. (1984). 20 μg bovine growth hormone (dissolved in 50 μl 0.05 M borate buffer, pH 9.3) was mixed with 2 μg fluorescein isothiocyanate (dissolved in 50 μl borate buffer, pH 9.3) in a microcentrifuge tube for 4 h at room temperature in the dark. The reaction was stopped by addition of 100 μl termination buffer (2 M glycine, 0.05% BSA, 0.05% sodium azide and 0.05 M borate buffer, pH 9.3). The reaction mixture was then dialysed against PBS (pH, 7.2) for 24 h at 4°C. Four changes of dialysand (PBS) were made during 24 h of dialysis. The growth hormone labelled with fluorescein was stored at -20°C in the dark in small aliquots until use.

Preparation of biotin labelled growth hormone

Biotinylation of bovine growth hormone was carried out as per the method described by Harlow and Lane (1988). 100 μl growth hormone solution (20 μg/ 100 μl 0.1 M borate buffer, pH, 9.0) was added to a 1.5 ml eppendorf tube. 4 μl of biotinamidocaproate-N-hydroxysuccinimide ester (20 μg) was then added and the mixture was thoroughly mixed. The tube was incubated for 4 h at room temperature. The coupling reaction was stopped by addition of 8 μl termination solution (0.25 M ammonium chloride in 0.5% BSA). After 30 min., the entire content of the tube was dialysed against PBS (six changes) for 48 h at 4°C and stored at -20°C in small aliquots.

Collection of adipose tissue

Adipose tissue was collected from the peritoneal region of slaughtered buffalo calves (1.5 years old, male). The tissue was placed in sterile saline (0.158 M NaCl, 37°C) containing 100 μg/ml streptomycin and 100 IU/ml penicillin (SSP). The tissue was then washed three times with SSP solution. For this purpose, three wells of a 6-well plate were half filled with SSP solution and adipose tissue was placed in one such well for 30 sec. and then transferred to the next well. This process was repeated twice and the tissue was finally transferred to a polypropylene bottle containing SSP solution and brought to the laboratory.

Localization of growth hormone receptor

Isolation of cells from adipose tissue: Cells from adipose tissue were liberated by treating the tissue with collagenase as per the method of Sato et al. (1980) with some modifications. Adipose tissue (500 mg) was washed with DMEM medium (37°C) and placed in a petri dish containing 10 ml digestion medium (DMEM medium containing 2 mg/ml collagenase, 30 mg/ml BSA, 100 μg/ml streptomycin and 100 IU/ml penicillin). The tissue was minced, placed in a 25-cm^2 tissue culture flask and incubated at 37°C in a water bath. The tissue was gently shaken during incubation. Within 15-20 min., cells started liberating from tissue. The digested tissue was filtered through four layers of cheese-cloth. Undigested tissue fragments and debris were retained on the cheese-cloth and discarded. The filtrate containing mature adipocytes and stromal-vascular cells was collected in 15 ml centrifuge tubes. The top layer contained mature adipocytes (Rodbell, 1964) and was collected by a 1 ml tip (in conjunction with dispenser) whose end was made 3 mm shorter with a sharp-edged blade. The mature adipocytes were transferred to a polypropylene tube containing 10 ml DMEM medium supplemented with 2% BSA, 100 μg/ml streptomycin, and 100 IU/ml penicillin (37°C). This washing step was repeated twice. The top layer containing mature adipocytes was used for localization of growth hormone receptor.

Incubation of adipocytes with fluorescein-labelled growth hormone

Equal volume of adipocyte suspension and fluorescein-labelled growth hormone were mixed for 5 min. at 37°C in the dark in a 0.5 ml microcentrifuge tube. Adipocytes, being lighter, formed a white layer at the top surface of the solution. These were gently collected with the help of a 200 μl plastic tip connected to a dispenser whose end was made shorter with a sharp-edged blade. These adipocytes were then placed in another centrifuge tube and washed with DMEM medium (37°C) two times to remove unbound labelled growth hormone. The washings were treated in a similar way by collecting adipocytes from the top layer and placing them in a new microcentrifuge tube containing DMEM medium. These adipocytes were subsequently transferred to a petri plate and observed under a fluorescence microscope using a blue filter and photographed using an automatic Olympus PM10SP photomicrographic system.

Determination of affinity constant and receptor sites

A new approach was employed for determination of the affinity constant for binding of growth hormone to its receptor present on adipocytes. It was not possible to obtain free-floating adipocytes, since such cells from buffalo adipose tissue aggregate quickly during handling and incubation, so affinity values were determined with explants. Buffalo adipose tissue was maintained for 2 days in DMEM medium containing 1 mM phenylmethyl sulfonyl fluoride (PMSF) at 37°C in a 5% CO_2 environment. Adipose tissue explants weighing 20 mg (wet weight after removing excess medium) were washed at least three times with PBS containing 0.1% BSA and 1 mM PMSF. Five explants of 20 mg each were placed in the wells of a 6-well-culture plates. 3 ml biotinylated GH (3.72 μg, final concentration 48.6 nM) and 500 μl of variable concentrations of unlabelled GH (final concentration in different wells was 0, 10^{-9} M, 10^{-8} M, 10^{-7} M, 5×10^{-7} M, 10^{-6} M, 5×10^{-6} M and 10^{-5} M) were added to each well. The explants were incubated for 2 h at 37°C with occasional shaking. Explants from each set were removed and quickly washed four times with PBS containing 0.1% BSA and 1 mM PMSF. After washing, 1.4 ml diluted streptavidin-peroxidase conjugate (1:14,000 with PBS containing 0.1% BSA and 1 mM PMSF) were added to explants from each set and incubated at 37°C for 15 min. These explants were again quickly washed five times with PBS containing 0.1% BSA and 1 mM PMSF. All five explants from each set were then separately incubated with 1.5 ml substrate solution (4 mg o-phenylene diamine hydrochloride dissolved in 10 ml 50 mM citrate buffer ,pH 5.0 containing 3.3 μl of 30% H_2O_2). The enzymatic reaction was terminated after 15 min. by addition of 1.5 ml 4 N H_2SO_4. The supernatant was decanted and absorbance was recorded at 490 nm.

Since absorbance data were to be converted into concentration of bound-GH, it was essential to establish this relationship. For this purpose, 2 μl biotinylated GH (0.35 μg) was dot blotted on 0.5×0.5 cm^2 nitrocellulose membrane and was blocked by 1% BSA (2 h, room temperature). The blocked membrane was treated with 50 μl diluted (1:1,000) streptavidin-peroxidase conjugate. The membrane was washed five times with PBS. Then, 1.5 ml of substrate solution was added. After 15 min., 1.5 ml 4 N H_2SO_4 was added to stop the reaction. Absorbance of the solution was recorded at 490 nm.

The affinity constant (Ka) and number of receptor sites per mg of tissue (Ro) were calculated by employing following Scatchard equation:

(Bound hormone)/(Free hormone) = $Ka\times(R_o)-K_a\times$ (Bound Hormone) (Scatchard, 1949). Bound growth hormone concentrations were calculated after subtracting non-specific binding which in the present experiment was obtained at 502.7 nM bGH concentrations. The ratio of bound (B) to free (F) was calculated and plotted against bound growth hormone (B) concentrations.

Identification of signaling molecules

Incubation of explants with growth hormone and preparation of lysate: Each adipose explant (100 mg) was maintained in DMEM medium without FCS for 24 h in a 5% CO_2 environment. After 24 h, adipose tissue explants were transferred to a 6-well tissue culture plate and washed with fresh DMEM medium. These explants were then transferred to 15 ml polypropylene tubes and 2 ml of GH (4.5 nM in PBS) solution was added. The tubes were placed in a CO_2 incubator maintained at 37°C and a 5% CO_2 environment for different lengths of time (5 min., 30 min. and 60 min.). The polypropylene tube was removed from the incubator and immediately 2 ml ice-chilled lysis buffer was added. Lysis buffer contained 100 mM Tris, pH 7.4, 1% triton X-100, 1 mM PMSF, 1 mM Na-orthovanadate, 10 mM EDTA, 16 μM antipain, 22 μM leupeptin and 14.5 μM pepstatin and was prepared according to method of Foxwell et al. (2000). The explants were subjected to homogenization for 30 sec at 4°C and centrifuged (10,000× g, 45 min., 4°C). The pellet was discarded and the supernatant was collected. The supernatant was further centrifuged twice (10,000×g, 45 min., 4°C) to remove any possible sediment. Supernatant was aliquoted in small volumes and stored at -70°C.

Tissue lysates of adipose tissue explants untreated with GH were also prepared and stored in similar way.

Separation of tissue lysate proteins in SDS-PAGE and their transfer on PVDF membrane: Protein from tissue lysates was separated in SDS-PAGE by the method of Laemmli (1970). Proteins from the acrylamide gel were elctrophoretically transferred onto PVDF membrane as described by Towbin et al. (1979).

Visualization of signaling molecules by enhanced chemiluminescence's technique: After transfer of proteins, PVDF membrane was blocked by gently shaking membrane in 15 ml blocking solution (3% BSA in TBST) for 1 h at room temperature. TBST solution contained 20 mM tris (pH, 7.6), 0.136 M NaCl and 0.1% tween-20. The blocked membrane was washed with 5 ml TBST (2×2 min). The membrane was then treated with primary antibodies (antiphosphotyrosine mouse monoclonal antibody or anti-phosphoJak2 antibody raised in rabbit) for 2 h at room temperature. These antibodies were diluted 2,000 times (antiphosphotyrosine) or 1,500 times (antiphosphoJak2) with TBST solution containing 0.2% BSA before use. The membrane was then washed with 5 ml TBST (3×15 min). The membrane was treated with diluted secondary antibody- peroxidase conjugate for 2 h at room temperature. Secondary antibody for antiphosphotyrosine was anti-

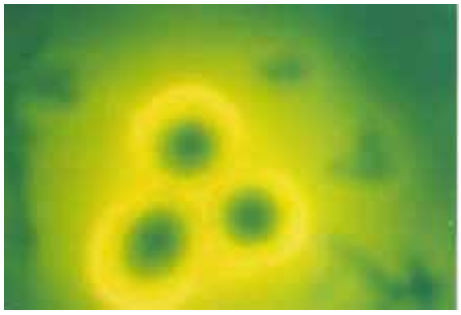

Figure 1. Visualization of growth hormone receptor on cell surface of buffalo adipocytes.

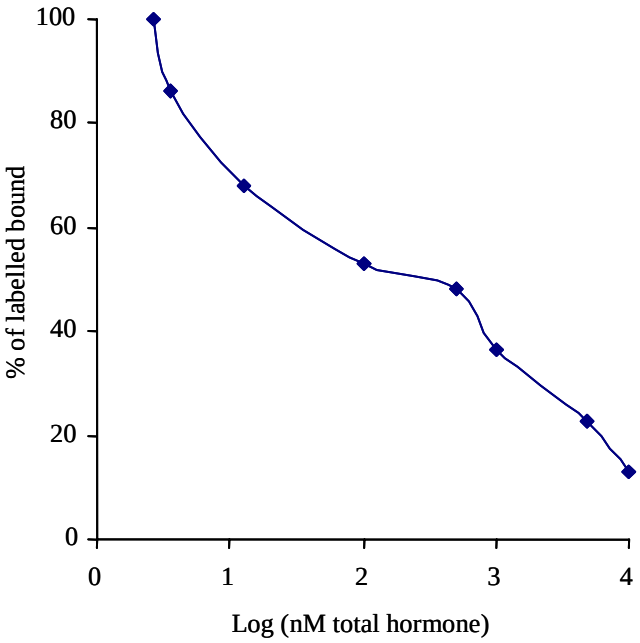

Figure 2. Binding of biotinylated-bGH to buffalo adipose tissue explants incubated with varying concentration of hormone.

mouse immunoglobulin (raised in rabbit). For antiphosphoJak2, secondary antibody used was antirabbit immunoglobin (raised in goat). Both secondary antibody-conjugates were diluted 500 times with TBST containing 0.1% BSA before use. The membrane was then washed with 5 ml of TBST (3×15 min) at room temperature. Chemiluminescence signal in the membrane was generated using an ECL Plus kit (Amersham Pharmacia Biotech) following the procedure recommended by the manufacturer. Autoradiography was performed on X-ray film.

RESULTS

Visualization of growth hormone receptor

When mature adipocytes were treated with FITC-labelled bGH and observed under fluorescence microscope, intense fluorescence on the surface of adipocytes was observed, suggesting presence of growth hormone receptor

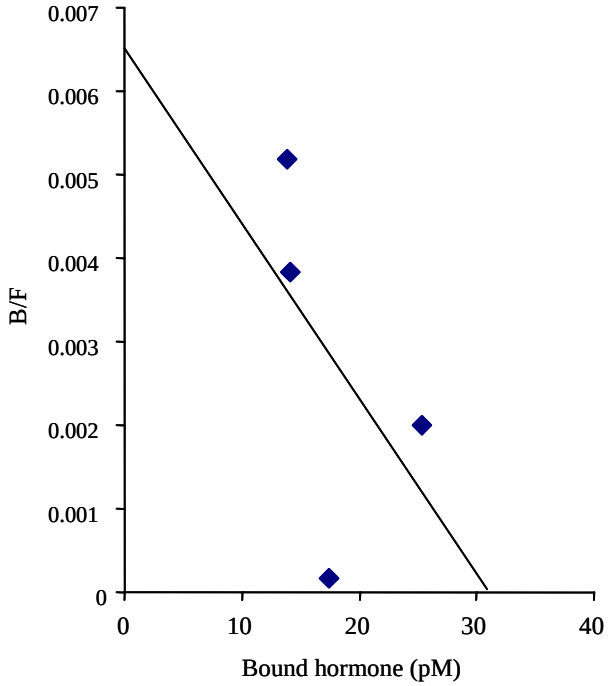

Figure 3. Scatchard plot of bGH binding to buffalo adipose tissue.

on the cell surface. The intensity of brightness of fluorescence indicated that the receptors were present in abundance (Figure 1). During isolation of mature adipocytes, at times ghost cells (spindle shape cells lacking fat) are formed and these too exhibited fluorescence (not shown in figure). This showed that growth hormone receptor did not dislodge from membrane under the conditions where the cell lost its fat.

Hormone receptor assay : For evaluating affinity of growth hormone to its receptor, a hormone receptor assay suitable for ruminant adipose tissue was developed. For validating the developed assay system, replacement of bound hormone was evaluated. For this purpose, increasing variable concentrations of (10^{-9}, 10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6}, 5×10^{-6}, 10^{-5} M) of unlabelled bGH were used in the incubation medium and binding of labelled GH was calculated as percent binding of labeled bGH without addition of unlabelled GH. The results of replacement of labeled bGH are presented in Figure 2. A sharp decline in binding of labeled bGH was observed in a GH concentration range of 2.7 nM to 102.7 nM. Further increase in concentration to 502.7 nM did not cause any additional significant decrease in binding of labeled bGH. In this range, it appeared as if saturation of bound labeled bGH had been reached. A further increase in concentration beyond 502.7 nM resulted in further replacement of bound labeled GH. In the range 502.7 to 10,002.7 nM, the replacement was linear with respect to increase in concentration of bGH. From these results, it appears that binding of labeled GH *vis a vis* total growth hormone concentration has two components, one component is

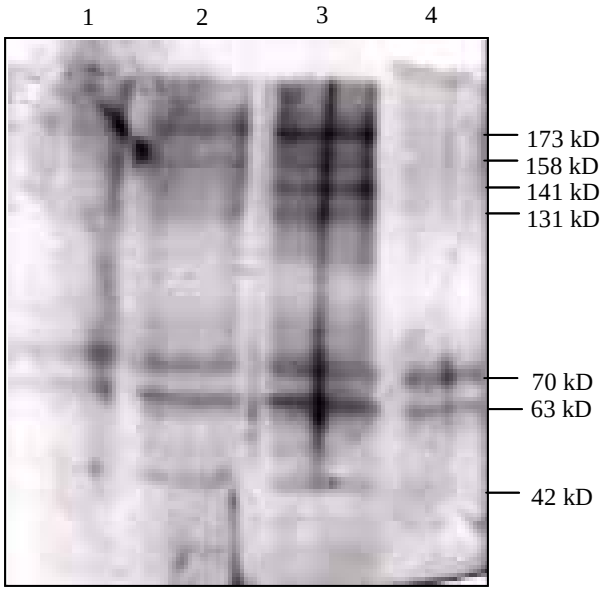

Figure 4. Identification of tyrosine phosphorylated proteins in buffalo adipose tissue treated with bGH. 5 µg tissue-protein was loaded in each lane of 7.5% SDS-PAGE. Phosphorylated proteins were detected on PVDF membrane (see methods). Explants without bGH treatment (Lane 1). Explants treated with bGH for 5 min. (Lane 3), or 30 min (Lane 2) or 60 min (Lane 4). Molecular weights of major bands are indicated.

saturable at about 100 to 500 nM bGH concentration while the other component is not. From the plot of bound hormone (B) to free hormone (F), affinity constant (K_a) and receptor concentration (Ro) in buffalo adipose tissue for bGH were found to be 2.3×10^8 M^{-1} and 1 fmole/mg tissue respectively (Figure 3).

Identification of signalling molecules based on their reactivity to anti-phosphotyrosine antibody : Mobility of proteins in SDS-PAGE is one of the useful methods for predicting identity of proteins. However, if a sample contains numerous proteins, as in case of cell lysate, it is difficult to identify proteins having similar molecular weights. In such a situation, reagents which could specifically interact with some of the separated proteins are very useful for predicting identity of the proteins. Signalling molecules are present at very low concentrations and are activated (phosphorylated) transiently. Tyrosine-phosphorylated proteins were reacted with anti-phosphotyrosine antibody in Western blot on PVDF membrane and subsequently detected by enhanced-chemiluminescence as described in the methods. A number of proteins reactive to antiphosphotyrosine antibody were observed when buffalo adipose explants were treated with 4.5 nM bGH for different periods (5 min, 30 min and 60 min). When buffalo explants were treated with bGH for 5 min., six bands of high intensity with molecular weights 173, 158, 141, 131, 70, 63 kD and one band with moderate intensity with molecular weight 42 kD were observed (lane 3 of Figure 4). During treatment of buffalo adipose tissue explants for 30 min. five bands with molecular weights 173, 158, 70, 63 and 42 kD were observed. These bands were of low to moderate intensity (lane 2 of Figure 4). Only two bands of low intensity with molecular weights of 70 and 63 kD were observed when explants were treated for 60 min. (lane 4 of Figure 4). The intensity of these tyrosine-phosphorylated proteins was compared visually and is presented in Table 1. Values of calculated molecular weights of bands and known molecular weights of signalling molecules were used in tentatively identifying the signalling molecules. As exposure time increased from 5 min, the number of bands declined. Exposure for 30 min resulted in disappearance of two bands and further exposure for 30 min (total exposure for 60 min) resulted in disappearance of three more bands. When tissue explants were not exposed to bGH, three bands of low intensity were also observed. From these results, it can be speculated that three signalling molecules, viz. insulin receptor substrate (IRS, Mol. Wt. = 165-185 kD), Janus activated kinase 2 (Jak2, Mol wt. = 120-140 kD) and mitogen activated protein kinase (MAP, mol.wt. = 42-44 kD), were activated during incubation of buffalo explants with bGH. These activated signalling molecules appeared in maximum concentration when explants were treated with bGH for 5 min.

Identification of Jak2 signalling molecule : Anti-phosphotyrosine antibody reacts with all proteins containing phosphorylated-tyrosine residues. These proteins are present in cell lysate which makes identification of activation of signalling molecules difficult. Therefore, specific antibodies against signalling molecules are preferred. Activation of Jak2 signalling molecule was

Table 1. Identification of tyrosine phosphorylated protein in buffalo adipose tissue reacted with bGH

Sample	MW (kD) of tyrosine phosphorylated proteins							Signalling molecules*
	173	158	141	131	70	63	42	
A	+	ND	ND	ND	+	+	ND	IRS
B5	+++	+++	+++	+++	+++	+++	++	IRS, Jak2, MAP
B30	+	+	ND	ND	++	++	+	IRS, MAP
B60	ND	ND	ND	ND	+	+	ND	None

* Tentatively identified; A represents explants not-treated with bGH; B5 represents explants treated with bGH for 5 min; B30 represents explants treated with bGH for 30 min; B60 represents explants treated with bGH for 60 min; + represents low intensity band; ++ represents moderate intensity band; +++ represents high intensity band; ND stands for not-detected.

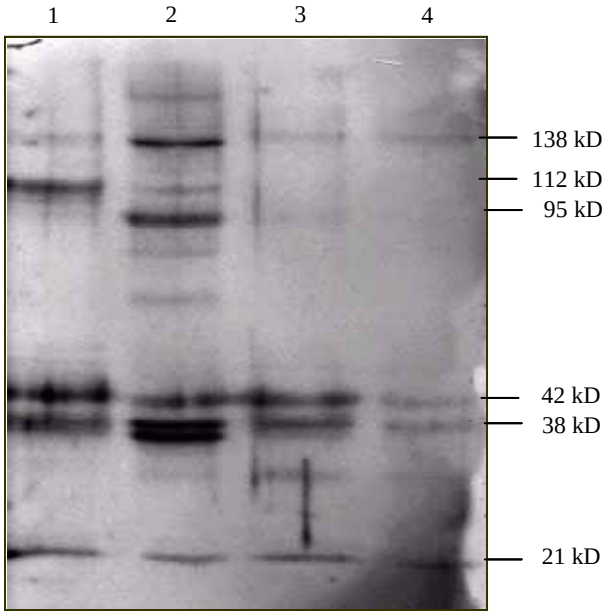

Figure 5. Jak2 activation in buffalo adipose tissue. Explants were incubated with 4.5 nM bGH for different period. 5 µg tissue-proteins were loaded in each lane of 6% SDS-PAGE. Phosphorylated Jak2 was detected on PVDF membrane (see methods). Explants without bGH treatment (Lane 1). Explants treated with bGH for 5 min (Lane 2), or 30 min (Lane 3) or 60 min (Lane 4). Molecular weights of major bands are indicated.

identified by employing anti-phosphoJak2 antibodies. Figure 5 shows the profile of protein bands detected by employing anti-phosphoJak2 antibody and enhanced chemiluminescence on PVDF membrane from buffalo tissue explants exposed to 4.5 nM bGH. When buffalo tissue explants were treated with bGH for 5 min., five bands with molecular weights 138, 95, 42, 38, 21 kD were observed (lane 2, Figure 5). These bands were of moderate to high intensity. One of the bands at 38 kD position was observed as a doublet (lane2, Figure 5). Three bands of low intensity (138, 95 and 21 kD) and two bands of high intensity (42 and 38 kD) were observed when buffalo adipose tissue explants were treated with bGH for 30 min. (lane 3, Figure 5). Exposure of tissue explants with bGH for 60 min. resulted in four bands (138, 42, 38 and 21 kD) of low intensity (lane 4, Figure 5). The intensity and generation of anti-phosphoJak2 reactive proteins in tissue lysates of buffalo adipose treated with bGH for varying periods is presented in Table 2. These results suggested that Jak2 molecule activation occurred in 5 min of exposure of explants with bGH.

DISCUSSION

The present work demonstrated the presence of bGH receptor on the surface of buffalo mature adipocytes. The high intensity of fluorescence indicates that receptors are present in abundant numbers on the cell surface. Localisation of receptors using FITC-bGH is simple and can be adopted without difficulty. Since FITC-bGH interacted with buffalo adipocytes, bGH must have a similar structure to that of buffalo GH. Buffalo GH is identical to bGH (Mukhopadhyay, 1999). Because of this fact and non-availabilty of buffalo GH, bGH has been used in the present work. Since yield of single cell preparations of mature buffalo adipocytes has been too low to allow determination of affinity constant, this constant was determined with buffalo tissue explants. From the replacement of labeled bGH, it is clear that binding of GH to the receptor has two components: one component is saturable at 100-500 nM bGH concentration and other component is non-saturable at more than 500 nM concentration (Figure 2). The affinity constant (Ka) is 2×10^{8} M^{-1} and is slightly lower than reported by others for bovine (Akers and Keys, 1984). In the present work, a new approach for measurement of Ka was used where radioisotope (I^{125}) has been omitted and binding of bGH was measured in terms of peroxidase activity. The method is simple to perform and does not require a gamma counter and radioisotope. However, sensitivity of biotin labelled bGH is far lower than radiolabelled GH. Therefore, biotinylated bGH in nM concentrations needs to be added for obtaining a measurable signal in the hormone receptor assay.

Identification of signalling molecules generated consequent to interaction of bGH with receptor is essential in order to understand the biochemical pathways operated by GH action. The level of signalling molecules is low and cannot be detected by conventional methods. However, for enhancing the sensitivity of the detection method, coupled reactions are used. In the present investigation, anti-

Table 2. Identification of activation of Jak2 in buffalo adipose tissue reacted with bGH

Sample	MW (kD) of tyrosine phosphorylated proteins						Signalling molecule
	138	112	95	42	38	21	
A	ND	+++	ND	+++	+++	++	-
B5	+++	ND	+++	+++	+++	++	Jak2
B30	+	ND	+	+++	+++	++	-
B60	+	ND	ND	+	+	+	-

A represents explants not-treated with bGH; B5 represents explants treated with bGH for 5 min; B30 represents explants treated with bGH for 30 min; B60 represents explants treated with bGH for 60 min; + represents low intensity band; ++ represents moderate intensity band; +++ represents high intensity band; ND stands for not-detected.

phosphotyrosine antibody interacted with phosphorylated tyrosine present in activated signalling molecules and sensitivity was enhanced by ECL. The sensitive methods are prone to provide more background and non-specific bands were observed with buffalo adipose tissue explants. Tyrosine phosphorylation is often the earliest detection event in GH signal transduction. Based on it, activation of three signalling molecules viz. IRS, Jak2 and MAP has been observed. In the detection system employing anti-phosphoJak2 antibody, the activation of Jak2 in the action of GH in buffalo adipose tissue has been confirmed. The intensity and pattern of bands established that Jak2 is activated in 5 min. exposure of buffalo adipose tissue with bGH. Since, in resting cells Jak2 is not activated no band around 120-140 kD was observed. Jak2 activation has been established in a number of cell lines including rat fibroblast cell line 3T3F442A (Argetsinger et al., 1993; Argetsinger et al., 1995; Smit et al., 1997), human cell line HT1080 (Han et al., 1996), human lymphocyte cell line IM-9 (Gurland et al., 1990; Finbloom et al., 1994; Frank et al., 1994; Silva et al., 1994), Chinese hamster ovary cell line CHO (Argetsinger et al., 1995; Billestrup et al., 1995; Vaderkuur et al., 1995) and *in vivo* in the rat (Chow et al., 1996; Ram et al., 1996; Thirone et al., 1999). An isoform of Janus activated kinase, Jak1, is absent in IM-9 (Gurland et al., 1990; Finbloom et al., 1994; Frank et al., 1994; Silva et al., 1994) and CHO (Argetsinger et al., 1995; Billestrup et al., 1995; Vaderkuur et al., 1995) but present in 3T3F442A (Argetsinger et al., 1993; Argetsinger et al., 1995; Smit et al., 1997). It appears that perhaps activation of Jak2 occurs in most cell lines/tissue by the action of growth hormone. The activation of insulin- receptor substrate (IRS) and mitogen activated protein (MAP) kinase has been reported in 3T3F442A (Argetsinger et al., 1993; Argetsinger et al., 1995; Smit et al., 1997) and CHO (Argetsinger et al., 1995; Billestrup et al., 1995; Vaderkuur et al., 1995) cell lines. These signaling molecules, besides Jak2, are activated in buffalo adipose tissue in the first five minutes of contact of tissue with growth hormone. The activation of Jak2 in buffalo adipose tissue is unequivocally established and it can be concluded that the initial step in activation of signaling molecules in buffalo (a ruminant) is similar to that in non-ruminants.

Jak2, a 130 kD tyrosine kinase, is the first signaling molecule activated as a result of binding of GH to its receptor (Silvennoinen et al., 1993). The activation of Jak2 occurs from autophosphorylation at the tyrosine side chain. The activated Jak2 subsequently phosphorylates GHR tyrosine residues present in the cytoplasmic domain. The binding of activated Jak2 with GHR is the first interactive event in the cytoplasm that activates other signaling molecules such as IRS, MAP and signal transducers and activators of transcription (Stat) proteins (Frank et al., 1994).

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