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CONTROL IN CELLULAR ACTIVITY BY INTERACTION OF PEPTIDES.

A. A. UMAR DIKKO AND ISA MARTIN IYORTIM

Department of Hhuman Physiology. Faculty of Medicine Bayero, Kano.

Summary: The interaction between compounds of similar structure has been studied, and often provides crucial information on the identity of the receptor subtypes. The information obtained can be applied in therapeutic and preventive medicine. An experiments was conducted in the previous years using EGF, PTH-rP and PTH(1-34) to investigate the interaction between these peptides on the proliferation of JAR human chariocarcinoma cells. Here the interaction between some of the fragments of hypercalcaemic factor PTH-rP and PTH(1-34) were considered with the view to strengthening our earlier argument that PTH-rP and PTH in JAR cells have a receptor which differs from the classical type I receptor present in osteosarcoma SaOS-2 cells and on the other hand to show that fragment of the same or similar compound, could interact with the father peptide and antagonize or agonise its action as this may be one of the methods cells control proliferation, differentiation and other functions. On the other hand it is possible that PTH(1- 34) and PTH-rP(1-34) compete on the PTH/PTH-rP receptor in JAR since effects of PTH(1-34) were only observed at higher concentrations. Potential antagonist action of PTH-rP(7-34) and PTH(1-34) on PTH-rP(1-34) and PTH-rP(186) stimulated cell proliferation were investigated using cell proliferation and DNA assay as an end point. It was found that JAR choriocarcinoma may not have the same receptor as in SaOS-2 and that PTH-rP(7-34) and PTH(1-34) can regulate cell proliferation. Furthermore, fragments of the same peptide may act in an opposing manner providing an insight on how cellular functions are regulated.

Key Word: *Parathyroid Hormone related peptide (PTH-rP),Parathyroid Hormone (PTH) and Epidermal Growth Factor (EGF).*

Introduction

The interaction between compounds of similar structure has been studied, and often provides crucial information on the identity of the receptor subtypes. The information obtained can be applied in therapeutic and preventive medicine. Umar and Peddie (1997) conducted experiments in the previous years using EGF, PTH-rP and PTH (1-34) to investigate the interaction between these peptides on the proliferation of JAR cells. Here we consider the interaction between some of the fragments of the hypercalcaemic factor PTH-rP and PTH(1-34) with the view to strengthening our earlier argument, that PTH-rP and PTH in JAR cells have a receptor which differs from the classical type I receptor present in osteosarcoma SaOS-2 cells, and on the other hand to show that fragment of the same compound could interact with the father peptide and antagonize or agonise its action, this as a result could be one of the methods cells control proliferation and other functions.

PTH-rP(7-34) antagonizes the action of PTH-rP(1-34) on COS-7 cells (Chorev, at. al. 1990). PTH-rP(7-34) inhibited the hypercalcaemia induced by synthetic PTH-rP(1-34) in nude mice bearing PTH-rP secreting tumours indicating that this analogue has the ability to antagonize PTH-rP(1-34) in vivo (Nagasaki, et. al. 1989). Also PTH-rP(7-34) and PTH-rP(8-33) inhibit cAMP production (Tanaka, et. al. 1996) in a dose dependent manner. However, Safadi et al. 1995 showed PTH-rP(7-34) to have no effect on the proliferation of three cell lines; JAR, MCF 7 and SaOS-2, between the concentration range of 1.25-5nM.

Furthermore, the same authors argued previously that PTH-rP(1-34) had a different receptor with PTH-rP(1-34) in JAR cells. Thus, different fragments of PTH-rP and PTH stimulate opposite actions on the same cells (Behar, et. al. 1996). It is possible that PTH(1-34) and PTH-rP(1-34) compete on the PTH/PTH-rP-receptor in JAR since effects of PTH(1-34) were only observed at higher concentrations. However, the potential antagonist effect posed by peptides such as PTH-rP(7-34) and PTH(1-34) was investigated this provided a lee way by which normal cells regulate the extent of their proliferation which otherwise momentarily acting in someway like malignant but spatially controlled (Ohlsson, 1987). However, the control often goes out of

hand leading to the production of malignant cells that in lucky circumstances arrested and give way to benign swellings and removed using surgery.

Material and Methods.

Cell Culture Method.

JAR cells were grown to 80% confluence in defined media (RPMI) supplemented with 10% fetal calf serum, 2mm L-glutamine, 1% HB(1320mg. Oxaloacetic acid, 80mg insulin/100ml.) and 1% PSA. They were growth arrested for 24hr prior to any experiment by replacing with serum free medium. Cells were trypsinised {(10% trypsin EDTA in calcium/magnesium free Hank's Balanced Salt (HBS)} from the culture flasks and set up in 24 well plates at an initial density of 0.5µg DNA/well. PTH-rP(1-34) and PTH-rP(1-86) with or without PTH-rP(7-34)/PTH(1-34) on the proliferation of choriocarcinoma (JAR) cells. Proliferation was assessed using the DNA assay. Results are expressed as % control for each experiment, when untreated cells represent 100% control values. Data are M+/- SEM of seven experiments. Data were analysed by Student's Test.

DNA Assay

This technique was first used in 1979 by Labarca and Paigen, and is based on the enhancement of fluorescence seen when bis-Benzamide binds to DNA. This assay could be used to detect as little as 10ng DNA. 0.05M Na_2 HPO4 (3.549g.) were dissolved in 500ml analar water, and titrated against 0.05M Na_2 HPO4 (3.001g.) also dissolved in 500ml analar water and adjusted to pH 7.4 and a total volume of ~800ml. 2M NaCl molecular biology grade 116.88g per litre was added, and dissolved. The pH was adjusted to 7.4 using NaOH or HCl, and volume made up to 1l with analar water, and stored at 4°C. Bis-benzamide was prepared from stock (2mg in 10ml analar water) and stored at 4°C, and was diluted immediately before use with DNA assay buffer to 1:100. Standards were prepared, using 1ml volumes of calf thymus DNA. Standard solutions of 0.5, 1.0, 2.0, 4.0, and 8.0µg/ml concentration were prepared in DNA assay buffer. Three tubes each containing 100µl of cells from cell culture were sonicated with 900µl DNA assay buffer for 5min. and 1ml diluted BIS was added to make the total 2ml in all the eight tubes. Each tube's contents were transferred into cuvettes. The fluorescence was read at 342nm and 458nm excitation and emission.

Results

Proliferation was assessed using the DNA assay. Results were expressed as % control for each experiment. Data are M+/- SEM of seven experiments. Data were analysed by Student's Test. The highest stimulation was obtained at 2.5nM for PTH-rP(1-34) and PTH-rP(1-86). PTH-rP(1-34) 0-5nM stimulated proliferation in JAR cells was inhibited from 125% (P<0.001) by PTH-rP(7-34) 2.5nM to 87% (P<0.001) below the control (Table 1).

Likewise, PTH-rP(1-86) 0-5nM stimulated proliferation in JAR cell was inhibited from 128% (P<0.001)by PTH-rP(7-34) 2.5nM to 95% (P<0.001) below the control (Table 1).

PTH-rP(1-34) 0-5nM stimulated cell proliferation in JAR was also inhibited from 126% (P<0.001) by PTH(1-34) 5nM to 94% below the control (Table 2).

Finally, PTH-rP(1-86) 0.5nM stimulated cell proliferation in JAR was also inhibited from 127% (P<0.001) by PTH(1-34) 5nM to 90% (P<0.001) below the control (Table 2).

PTH-rP(7-34) neither alone or with PTH(1-34) 5nM had any effect on JAR cell proliferation (Table 3). Lastly, PTH(1-34) stimulated cell proliferation in JAR to 15% above the control while it had no effect with PTH-rP(7-34) 2.5nM on the proliferation of JAR cells.

PTH-rP(1-34) and PTH-rP(1-86) stimulated proliferation in JAR signifying what Gardella et. al. 1995 ascerted that both amino and carboxylic terminal are important when it comes to the stimulation of PTH-rP-R.

Table 1: Effect of PTH-rP(1-34) and PTH-rP(1-86) with or without PTH-rP(7-34) on the proliferation of choriocarcinoma (JAR) cells

Control	Peptide	P level	Peptide +	P level
Peptides and cellular activity control				
100%	126%	P< 0.001	87%	P< 0.001*
100%	128%	P< 0.001	95%	P< 0.001**

* PTH-rP(1-34) ** PTH-rP(1-86)

Table 1 and 2 Effect of PTH-rP(1-34) and PTH-rP(1-86) with or without PTH-rP(7-34) on the proliferation of choriocarcinoma (JAR) cells.
Proliferation was assessed using the DNA assay. Results are expressed as % control for each experiment, when untreated cells represent 100% control values. Data are M+/- SEM of seven experiments. Data were analysed by Student's Test. The stimulated cell proliferation of PTH-rP(1-34) and PTH-rP(1-86) were significantly inhibited by PTH-rP(7-34).

Table 2: Effect of PTH-rP(1-34) and PTH-rP(1-86) with or without PTH-rP(1-34) on the proliferation of choriocarcinoma (JAR).

Control	PTH-rP	P level	PTH-rP(1-34)+ PTH(1-34)	P level
100%	126%	P< 0.001	94%	P< 0.001 *
100%	127%	P< 0.001	90%	P< 0.001 **

* PTH-rP(1-34) ** PTH-rP(1-86)

Table 2 Effect of PTH-rP(1-34) and PTH-rP(1-86) with or without PTH-rP(1-34) on the proliferation of choriocarcinoma (JAR).
Proliferation was assessed using the DNA assay. Results are expressed as % control for each experiment, when untreated cells represent 100% control values. Data are M+/- SEM of seven experiments. Data were analysed by Student's Test. The stimulated cell proliferation of PTH-rP(1-34) and PTH-rP(1-86) were significantly inhibited by PTH(1-34).

Table 3: Effect of PTH-rP(7-34) and PTH-rP(1-34) with or without vice-versa on choriocarcinoma (JAR) cells

Control	PTH-rP(7-34)	P level	PTH-rP(7-34)+ PTH(1-34)	P level
100%	106%	N.S	94%	N.S. *
100%	103%	N.S	90%	N.S. **

* PTH-rP (7-34) ** PTH (1-34) N.S. Not Significant.

Table 3 Effect of PTH-rP(7-34) and PTH-rP(1-34) with or without vice-versa on choriocarcinoma (JAR) cells.

Proliferation was assessed using the DNA assay. Results are expressed as % control for each experiment, when untreated cells represent 100% control values. Date are M+/- SEM of seven experiments. Data were analysed by Student's Test. The increase in proliferation was not significant with PTH-rP(7-34) 0-5nM or with PTH(1-34) and PTH(1-34) 5nM, but significant.

Discussion

In the previous researches we tried to strengthen our hypothesis that JAR may not have a classical receptor by the use of PTH-rP and PTH on the proliferation of three different cell lines; JAR, MCF-7 and SaOS-2. We noticed that JAR cells show a different profile of responses from MCF-7 and SaOS-cells. It was suggested that the receptors signal transduction mechanism associated with them may differ. The possibility that the PTH-rP(7-34) and PTH(1-34) might have an antagonist effect on the stimulated cell proliferation of PTH-rP(1-34) and PTH-rP(1-86) in JAR cells, as well as an additional evidence that PTH-rP and PTH may have a different receptor were assessed.

PTH-rP(1-34) and PTH-rP(1-86) significantly increased cell proliferation in JAR. PTH-rP(7-34) inhibited the proliferation caused by PTH-rP(1-34) and PTH-rP(1-86) in JAR cells. These effects may be due to competition between the peptides on the receptor where PTH-rP(7-34) and PTH-rP(1-34) or PTH-rP(1-86) may compete to a similar location on the receptor; that is residue near the amino-terminus and within the third extracellular loop (Lee, et. al. 1994).

The different potencies that the two ligands exhibit with PTH/ PTH-rP-R receptor; residue 5(his in PTH-rP and Ile in PTH) that determines signaling capabilities (Phe in PTH-rP and Trp in PTH) the binding affinity. The 5th residue is found in PTH-rP(1-34) and 1-86 but not in PTH-rP(7-34). The competition between the PTH-rP(1-34) and 1-86 with PTH-rP(7-34) block the signal and inhibits proliferation. This has already been in part supported by Chorev, et. al. (1990), where these authors show PTH-rP(7-34) to antagonize the action of PTH-rP(1-34) on COS-7 cells.

On the other hand PTH(1-34) inhibited the stimulated PTH-rP(1-34) and PTH-rP(1-86) cell proliferation in JAR cells. This is

interesting because it suggests that PTH and PTH-rP(1-34) will bind to the PTH-rP receptor in JAR cells but does not activate it. This again could be explained by what Behar et, al. (1996) found in human embryonic kidney cells, where histidine in position 5 determines the receptor subtype i.e. PTH-rP(1-34) increased cAMP and cytosolic calcium, while PTH(1-34) devoid of the histidine in position 5 did not. Once again PTH(1-34) competes with PTH-rP(1-34) and PTH-rP(1-86) and blocks the signal transduction and proliferation. This strengthens our argument, that JAR/trophoblasts may have receptor which is different from the classical type I PTH-rP(1-34).

The significance of all these antagonist effect posed by peptides such as PTH-rP(7-34) and PTH(-34) is possibly a provision of a lee way by which normal cells regulate the extent of their proliferation which otherwise momentarily acting like malignant but spatially controlled (Ohlsson, 1987). However, the control often goes out of hand leading to the production of malignant cells that in lucky circumstances, arrested and give way to benign swellings.

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