STUDY ON EFFECT OF GHRP-6, AND PEPTIDES 96/185 AND 96/257 ON LH/FSH ACTIVITY

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ABSTRACT

GHRP-6 and ten of its analogues were evaluated for effect on the body weight gain after chromic administrations. Twenty one days old, juvenile female rats of Sprague Dawley strain were used for these studies. The rats were kept under standard laboratory animal house conditions and were provided water and food ad libitum. The animals were weighed at the beginning of the experiments and were divided into four groups with six rats in each. The compounds were freshly constituted prior to their administration and were given daily at 11 a.m. for fifteen days. Three different doses of 0.25, 1.0 and 3.0 mg/kg body weight were given subcutaneously. The control group of animals received an equal volume of normal saline. The body weight was measured on the alternate days using Fx-400. Afcoset balance. Percentage body weight gain was calculated at each dose level on the termination of the experiment.

Key words: *administrations, laboratory animal, compounds, termination.*

INTRODUCTION

Growth hormone (GH), a 191 amino acid long peptide in humans, is the most important hormone for the postnatal growth of body. It plays a major role as an anabolic factor and stimulates cellular growth and proliferation, growth of bones, muscles and connective tissue. Secretion of growth hormone is regulated by the two hypothalamic, viz., growth hormone releasing hormone (GHRH) and somatostain (SRIH), which stimulate and inhibit the GH secretion respectively. The under secretion of pituitary growth hormone (hypopituitarism) in childhood and adolescence leads to 'dwarfism' and over-secretion during this period causes 'gigantism'. Further, over-secretion of this hormone during adulthood after growth period, causes 'acromegaly'. It has been possible to investigate metabolic and other effects of GH with the availability of recombinant GH. Available results suggest potential and beneficial effects of GH therapy in osteoporosis cardiomyopathy, AIDS and other immune disorders.

REVIEW OF LITERATURE

The use of recombinant human growth hormone is not very ideal for treatment of growth hormone disorders, as it lacks oral bioavailability and also its efficacy to release GH is also questionable **(Coste et al, 1997)**. Hence, a host of compounds have been synthesized in the past two decades to obtain suitable and effective growth promoters. This exercise has been very rewarding and a peptide. GHRP-6 has been found to specifically stimulate GH release without affecting release of other pituitary hormones **(Bowers et al, 1984).**

GHRP-6 (His-D-Tra-Ala-Trp-DPhe-Lys-NH2), a hexapeptide, that has been developed by a combination of energy conformational calculations, synthesis, structure activity relationships of small peptides and biological activity assessment. **Bowers and his coworkers (1984)** were the first to describe both in vitro and in vivo activity of the hexapeptide.

It is now well established that GHRP-6 species of animals including human without any effect on the other pituitary hormones. Through, GHRP-6 is quite active but its oral efficacy is not sustainable for therapeutic. This promoted us to synthesis several analogues of GHRP-6 with higher receptor affinity and metabolic resistivity, these characteristics may favour the desired biological activity.

MATERIAL AND METHOD

Chronic studies with GHRP-6 and peptides 96/185 and 96/257.

It is known that GHRP-6 causes release of growth hormone without affecting the release of other anterior pituitary commoners. In the present study efforts have been made to study the effect of GHRP-6 and its potent analogues 96/185 and 96/257 on the ganadotropin functions.

Effects of GHRP-6 and its analogues 96/185 and 96/257 were studied on LH/FSH activity using the following parameters: vaginal opening, vaginal smear picture, uterine and ovarian weights. S.

D. female rats, 21 days of age were procured from the animals house of the institute and acclimatized for seven days in the laboratory at $25^0C \neq 2^0C$. Rats were divided into various groups and they received 0.3 or 1.0 mg/kg dose of GHRP-6, 96/185 or 96/257 subcutaneously for fifteen days. The control group received an equal volume of normal saline. On the sixteenth day vaginal opening was observed and vaginal smear was taken. This was followed by the animal autopsy to extract ovary and uterus. The weight of both the ovaries and uterus was taken. More comprehensive study was undertaken with the potent GHRP-6 analogues 96/257. This part of the study was performed using the superovulation method to assess the FSH and LH activity in immature rats. Twenty one days old SD female rats were divided into different groups consisting of three to five rats in each group. Rats were treated with a standardized dose of PMSG (50 IU) and hCG (30 IU) at an interval of 56 hrs. To asses degree of follicle maturation and subsequent ovulation. In order to assess FSH/LH like per se activity of peptides 96/257, it was substituted for PMSG/hCG or both in the protocol. In another two groups, rats were treated wither with PMSG or only hCG. A vehicle treated group was also run (Table 3). Within 24 hours after the hCG treatment, vaginal opening was observed and vaginal smear was taken in all the groups. Animals were autopsied to remove the uterus and ovaries. The organs were then cleaned, weighed and fixed in buffered formalin for 24 hours for histology. Oviducts were flushed properly using a very fine needle (30 gauge) and the number of ova were counted under the microscope after the addition of hyaluronidase enzyme in the flushing.

Table 3

Treatment Schedule of Compound 96/257 for The Acute Studies on Its effect on LH and FSH Release

S. No.	Day 1 (10.00 a.m)	Day 2 (10.00 a.m)	Day 3 (10.00 a.m)
1.	Vehicle	Vehicle	Vehicle
2.	PMSG (50 IU)	-	-
3.	-	-	hCG (30 IU)
4.	PMSG (50 IU)	-	hGH (50 IU)
5.	96/257 (0.3 mg/kg)	-	96/257 (0.3 mg/kg)
6.	96/257 (1.0 mg/kg)	-	96/257 (1.0 mg/kg)
7.	PMSG (50 IU)	-	96/257 (0.3 mg/kg)
8.	PMSG (50 IU)	-	96/257 (1.0 mg/kg)
9.	96/257 (0.3 mg/kg)	-	hGH (30 IU)
10.	96/257 (1.0 mg/kg)	-	hGH (30 IU)

The uterus and ovarian tissues collected in buffered formalin were dehydrated sequentially through 70% alcohol acetone benzene, paraffin wax and were finally embedded in paraffin was. Sections of 5 micro thickness were cut using microtome (Leica RM 2155 automaticmicrotome) and stained with haematoxyline and eosin. Then histological assessment was done under light microscope.

GHRP-6 and ten of its analogues were evaluated for effect on the body weight gain after chromic administrations. Twenty one days old, juvenile female rats of Sprague Dawley strain were used for these studies. The rats were kept under standard laboratory animal house conditions and were provided water and food ad libitum.

The animals were weighed at the beginning of the experiments and were divided into four groups with six rats in each. The compounds were freshly constituted prior to their administration and were given daily at 11 a.m. for fifteen days. Three different doses of 0.25, 1.0 and 3.0 mg/kg body weight were given subcutaneously. The control group of animals receivedan equal volume of normal saline. The body weight was measured on the alternate days using Fx-400. Afcoset balance. Percentage body weight gain was calculated at each dose level on the termination of the experiment.

CONCLUSION

The control group of animals received an equal volume of normal saline. Immediately after the injection the animals returned to their home cages and a weighed amount of food pellets were severed. The consumption of food was minored every hour up to a period of six hours by carefully weighing the remaining food top the nearest 0.001g. The food intake was then normalized, per 10g body weight of the mice. The effect of GHRP-6 and its analogues on the anorexia induced by an anorexigenic agent, amphetamine was also observed. Amphetamine was given subcutaneously at two doses of 2.5 or 5.0 mg/kg and 30 minutes later, the mice were placed in individual cages and exposed to sweetened milk for 15 minutes. Thereafter, the amount of milk left was measured to assess the level of anorexia.