8-P13

EFFECT OF ISOPROTERENOL, A NON-SELECTIVE β-AGONIST, ON LIPID METABOLISM OF RATS, : AN *In Vitro* AND *In Vivo* STUDY.

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Background

There are several systems involved in the incomings and outgoings of energy in animal body. When the energy is needed in the body, some of such systems such as glycolysis and lipolysis stimulate energy production through catabolic processes. Adrenergic system is considered as one of the important control system in such energetic processes of saccharides and lipids. In lipolysis, fats, mainly triglycerides, as one of the most important energy store are degraded into glycerol and fatty acids to produce a considerable amount of energy. It is believed that β-adrenergic receptors, mainly β-1, are involved in fat metabolism through the induction of lipolysis. Currently, β 3-adrenorecptor is found to be a key receptor in fat metabolism. Agonist stimulation of these receptors activates adenylate cyclase which converts ATP to cAMP. Produced cAMP acts as a "second messenger" by carrying information regarding lipolysis to intracellular sites.

Since a few decades ago, obesity has been considered as a major risk factor for some cardiovascular, metabolic and endocrine diseases. Therefore, many researchers have tried to manipulate fat metabolism for preventing excess fat accumulation in the body. Lipolysis may be considered as one of the potential ways.

There have been some studies indicating the possible interactions between dietary lipids and β-adrenergic lipolytic activities. It has been suggested that dietary lipids change the cell membrane composition and then affect adrenoceptor activity (For more details refer to authors' review paper).Our previous studies have shown some patterns of distribution and metabolism of different dietary intaked fats rich in saturated fatty acid (SFA), polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) in the rats body.

Objective: In this study, lipolytic effect of isoproterenol, a non-selective β-adrenoceptor agonist, has been studied in adipocyte culture and also possible interactions between the β-adrenergic activity and different dietary fats have been studied in rats raised on 4 different dietary lipids, including lipids with animal and vegetable origins.

Materials and Methods

Adipocyte culture and in vitro lipolysis study: About 1 mg of epididymal fat was dissected out from a 20 weeks old male SD rat and then adipocytes were isolated by collagenase digestion as previously described by Rodbell ,i.e. adipose fragments were incubated for 60 min in 5 ml Krebs-Ringer bicarbonate buffer containing albumin (3.5 g/100 ml) (KRBA), glucose (6 mM) and collagenase Ⅳ(3.5 mg/ml)at pH 7.4 and 37.5℃ in a water bath under gentle shaking at 60-70 cycle/min. After digestion, adipocyte suspension was centrifuged at 3000 g for 5 min and the supernatant was filtered through a 150 mesh stainless steel filter. Adipocytes were washed three times with KRBA buffer to eliminate collagenase. For lipolysis study, isolated adipocytes ($1\text{-}1.5\times10^4$ cells) were incubated in 1 ml KRBA (pH 7.4) containing glucose (6 mM) at 37℃ in polyethylene tubes containing 10 μM and 100 μM of isoproterenol hydrochloride (purchased from Sigma Co. Ltd.) with gentle shaking (60-70 cycles/min) in a water bath. After 90 min of incubation, the reaction was quenched by placing the tubes in ice bath and 100 μl of aliquots of infranatant were taken for enzymatic determination of glycerol (glycerol kit, Boehringer Mannheim GmBH) by using spectrophotometry (DU-62 Spectrophotometer, BECKMAN).

Animal study: Forty-eight 7-weeks old male SD rats (purchased from Seac Co. Ltd., Japan) were raised on commercial diet for one week for adaptation. Then, they were divided into 2 groups, i.e., one group as control and the other was treated with isoproterenol. Isoproterenol was dosed at 0.5 mg/kg body weight/day. Each group was further divided into 4 sub-groups fed diets containing 4 different fats; 12% of beef tallow, canola oil, olive oil or safflower oil. Other ingredients were the same for the four groups: 20% beef powder, 1% AIN-76 vitamin mixture, 3.5% AIN-76 mineral mixture, 0.3% DL-methionine, 0.2% choline bitartrate, 5% cellulose, 27.9% corn starch, 30% sucrose, 0.1% cholesterol. In beef tallow diet, 0.03% alpha-tocopherol (wt/wt) was added to beef tallow

itself before mixing with other ingredients, as an antioxidant. All rats have been raised on these diets and under controlled conditions (12 hr. light/day, 20℃ temperature and 60% relative humidity) for 1 month in separated cages in animal raising facilities of Biotron Institute of Kyushu University. Weight gain and feed intake were measured every other day. After 1 month raising, rats were anaesthetized by ethyl ether and killed (carried out under the control of guideline for Animal Experiment in Faculty of Agriculture and the Graduate Course, Kyushu University and the Law [No.105] and Notification [No.6] of the Government) and their abdominal fat mass and liver were dissected out and weighted. Fat content of abdominal fat, liver, thigh muscle and subcutaneous adipose tissue was extracted by Folch's method and their fatty acid composition was analyzed by gas chromatography (GC-14B, GAS CHROMATOGRAPH, SHIMADZU Co. Ltd., Japan).

Results

In-vitro lipolytic study: The concentration of glycerol was 22.23×10^{2} µmol and 16.72×10^{2} µmol/1-1.5×10^{4} cells in tubes incubated with 100 µM and 10 µM isoproterenol, respectively. Glycerol concentration of control, as an index of basal lipolysis was 10.54×10^{-2} µmol /1-1.5×10^{4} cell.

Animal study: Daily feed intake and weight gain was not significantly different among the groups of both control and agonist treated. Although the amount of abdominal fat was less in agonist-treated (11.928 g) than control (15.25 g) in beef tallow-intaked rats , the difference was not statistically significant. There was no significant difference in liver weight among the dietary groups and also between agonist-treated and control. Fatty acids composition of collected samples also showed no significant differences between agonist-treated and control dietary groups.

Discussion

Lipolytic activity of isoproterenol has been studied by several research groups, both *in vitro* in adipocyte culture and *in vivo* through infusion in human and animals. Some studies have shown that low concentration of catecholamine (10 nM or lower) activates lipolysis through β 1- adrenoceptor activation ,whereas the stimulation of the β 3-adrenoceptor occurred at higher concentration (more than 1µM). In this study, isoproterenol showed a dose-dependent lipolytic activity in rats adipocyte, possibly due to above mentioned mechanism. There have been some reports indicating that fatty acid composition of diet can affect adipocyte cell membrane composition. Matsuo et al. (1997) have shown that beef tallow diet promotes body fat accumulation due to reduced lipolytic activities resulted from lower-receptor binding and sympathetic activity in adipose tissues. In the present study, however, interaction of dietary fats and isoproterenol on the accumulation of fat in rat body was not significant. One of the main reasons of the non-significance might be less bioavailability of isoproterenol due to so-called "first-pass loss" by liver.

Conclusion

Although isoproterenol as one of the non-selective β-adrenergic agonist activated lipolysis in adipocytes in vitro, the lipolytic effect of the agonist was not significant in animal study. This might be because of non-selective agonist activities of isoproterenol, lesser lipolytic activity of β 1-adrenoceptor, compared to β 3-adrenoceptor, and also of lower concentration of the agonist bound to the adrenoceptors located on the surface of the adipocyte due to the reduction in agonist level after first-pass loss in the liver. Results of the present study also indicated that dietary lipids showed no significant effect on lipolytic activity, if any, of isoproterenol.

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