Adrenergic control of lipid metabolism; in vitro studies

Abstract

The difficulty with using whole fish is that catecholamines may alter the levels of other circulating hormones, and that catecholamine turnover in the circulation is rapid. Furthermore, the responses of the different fat storing tissues including the liver may be different. This problem is prevented when using isolated fat or liver tissue, or isolated adipocytes and hepatocytes. Migliorini *et al*. (1992) investigated lipolysis in adipose tissue slices of fish, toad, and snake. Remarkably, basal rates were much lower than those of mammals. Catecholamines had, however, no effect on lipolysis in fish and toad adipose tissue. Adrenaline decreased lipolysis in snake adipose tissue, while glucagon had a clear lipolytic effect on snake, both in-vivo and in-vitro. The release of FFA from all adipose preparations was stimulated by cAMP, xanthine derivatives and phosphodiesterase-inhibitors. Lipolysis was also stimulated by fluoride and forskolin, which also increased cAMP levels. These results, suggesting that lipolysis is mediated via phosphorylation of a lipase, did not support Farkas' idea that lipase is not regulated by cAMP . The advantage of working with isolated cells instead of chopped adipose tissue is that the influence of damaged cells is negligible, and that reproducible incubations from the same source can be made to study concentration dependent effects of different agonists and antagonists. To verify the hypothesis that β-adrenoceptor stimulation leads to inhibition of lipolysis in adipose tissue, we carried out experiments with isolated adipocytes from tilapia mesenteric fat tissue . The paper of Vianen *et al*. (2002) was the first to show in-vitro lipolysis in isolated fish adipocytes. Zhou *et al*. (1996) described the histology and size range of Atlantic salmon adipocytes. Christiansen *et al*. (1985) describing glucose uptake studies pointed to the fragility of adipocytes isolated from trout adipose tissue. Their adipocytes could not be incubated much longer than 4 minutes. The procedure of Vianen *et al*., however, allows incubations for 5 hours, in some cases overnight incubations were even performed. A general draw-back of working with lipolysis in fish adipose tissue appears the large variability in FFA release. The basal lipolytic rates in teleost fish range from 2-1500 nmoles/h/10^6 cells. In catfish, trout and seabream the basal lipolytic rates were about 650, 130 and 2 nmol/h/10^6 cells, respectively, whereas in tilapia values of about 1500 nmoles/h/106 cells were found . The values are dependent on age, season, nutritional condition, and also on the level of maturation. Basal lipolytic rates observed in mammals range between 50-150 nmol/h/10^6 cells. Published rates are: 65-100 nmol/h/10^6 cells for rat, 150 nmol/h/10^6 cells for sheep,

150 nmol/h/10^6 cells for pig, and 65 nmol/h/10^6 cells for human. These rates are relatively low compared with the values obtained from fish adipocytes especially in view of the higher incubation temperature (37°C) with mammalian adipocytes. On the other hand, β-receptor stimulation in mammals leads to a strong increase of lipolysis reaching maximum rates of 2.000-40.000 nmol/h/10^6 cells, which are 10-100 fold higher than those observed with teleosts. The paper of Vianen *et al*. (2002) confirmed that noradrenaline reduces the FFA release of adipocytes dose-dependently, which became significant at concentrations of ≥1μM. Co-incubation of noradrenaline with phentolamine (α1,2-antagonist) showed no effect, indicating that no α-adrenoceptor is involved. On the other hand co-incubation with timolol (β1,2-antagonist) showed that the anti-lipolytic effect of noradrenaline was caused by β-adrenoceptor stimulation in sharp contrast to the situation in mammals . We also found evidence for inhibitory β3-adrenoceptors; which are activated at concentrations of isoproterenol ≥10 μM and by the selective β3-adrenoceptor agonist BRL 35135 . A possible function for those receptors is a safety valve: at high catecholamine concentrations that are found during hypoxia lipolyic activity becomes arrested. This finding was the first report of a functional β3-adrenoceptors in fish. Thus far no reports are known of an inhibitory β3-adrenoceptor. Nickerson *et al*. (2003) cloned β-adrenoceptor genes of trout, and found that two were homologous with mammalian β3-adrenoceptors. In a detailed study they showed two subtypes in trout, a β3-a and a β3-b form. The first expressed predominantly in gill and heart, and the latter exclusively in erythrocytes. Binding studies suggested that the β3-adrenoceptor in erythrocytes is involved in sodium/proton exchange, the function of this adrenoceptor in the other tissues remains to be resolved. With respect to the β-adrenoceptors on adipocyte membranes, we could confirm with trout, catfish, and sea bream the inhibitory effect on lipolysis. This suggests a general mechanism in teleost fish. Contrary to the observations by Migliorini *et al*. (1992) with fat tissue from the adult male tigerfish, exposure of tilapia adipocytes to increasing concentrations of isobutyl-methylxanthine (IBMX) and forskolin resulted in a marked reduction of FFA-release. These results suggest a cAMP-dependent inhibition of TG lipase activity, which is the opposite from the transduction mechanism in mammals. The observation that Migliorini *et al*. (1992) found an increase in lipolytic activity may be due to the (very) high concentrations of IBMX and forskolin, which were applied, 10 and 0,1 mM, respectively. So, fish adipocytes have β-adrenoceptors which upon activation reduce lipolysis via a cAMP mediated mechanism. Liver slices of coho salmon incubated in vitro release fatty

acids upon stimulation by 1 μM noradrenaline but not by adrenaline. Also isoproterenol (β-receptor agonist) stimulated FFA release. The lipolysis occurred at a 3 to 1 ratio of FFA and glycerol respectively. The effects of noradrenaline and isoproterenol could be blocked by propranolol (β-receptor antagonist) but not by phentolamine (α-receptor antagonist), prazosin (α1- receptor antagonist) and yohimbine (α1-receptor antagonist). This shows that lipolysis in the salmon liver is activated through β-adrenoceptors. In a recent study with trout hepatocytes we could demonstrate that lipolysis is activated by β2-adrenoceptors.

CONCLUSIONS

There is overwhelming evidence that lipid mobilization is differently organized in fishes compared to mammals. In general it appears that lipolysis in mammals is strongly activated by catecholamines via β-adrenoceptors (figure 5), while in fishes lipolysis is mostly inhibited. In mammals some inhibition may occur via α2-adrenoceptors. The basal lipolytic rates in fish are sometimes barely measurable, which may compromise the effect of controlling hormones. This pattern is related to the changing needs for storage and mobilization in fishes; lipids are stored in different locations and mobilized differentially for maturation, migration, and long periods of starvation. The fat storage near and in red muscle forms a very short transport route to the muscle. This obscures FFA turnover measurements. The FFA concentrations per se do not provide an indication about lipolysis. However, assuming a fast response on catecholamine release, the fall in oxidation capacity due to oxygen shortage will be slower than the inhibition on HSL. Thus this will in general lead to a lowering of the plasma FFA levels.

The oxygen content of water is some 30 times lower than that of air, in addition oxygen diffusion rates in water are much lower than those in air (800 times), and this makes water-breathers very sensitive to changes in oxygen content. Actually water can become hypoxic or even anoxic in a few hours under certain conditions. Since hypoxia is a generally occurring phenomenon, it may be expected that fish are better adapted to hypoxia than mammals. Between fish species the tolerance to hypoxia varies greatly, and some species have developed "unorthodox" metabolic pathways and behaviour in order to survive extreme hypoxic/anoxic conditions. During normoxic conditions, lipids and amino acids are the major fuels for the energy metabolism in teleost fish whereas carbohydrates become important during anaerobic conditions such as burst activity and hypoxia. In mammals ischemia and severe hypoxia results in membrane damage. This

damage is mainly caused by the accumulation of amphiphiles such as fatty acids and the intermediates of β-oxidation. Fatty acids are amphipatic molecules and are known to destabilize biomembranes. The flux through β-oxidation is high, particularly in mammalian heart muscle during normoxic conditions. Due to inhibition of the lipid oxidation fatty acids and their metabolites will accumulate rapidly. In addition, hypoxia gives always rise to a massive release of catecholamines. In mammals these hormones cause a strong activation of lipolysis. Thus the combination of catecholamine-induced lipolysis and strongly reduced capacity to oxidize fatty acids results in a fast and major accumulation of fatty acids and intermediates of the β-oxidation. Severe ischemia and hypoxia may lead to a marked depletion of the phosphorylation potential resulting in ion rearrangements, in particular in Ca^{++}-influx. Increased Ca^{++}-influx results in turn in activation of phospholipases and consequently in membrane leakage. The above processes ultimately lead to cell damage and to leakage of intracellular enzymes (such as LDH and CK) into the circulation. While hypoxia in mammals is considered as a pathological condition, this is not the case for (water-breathing) fishes. Actually hypoxia/anoxia induced membrane damage does not occur in fish even after near lethal exposures. So obviously fish, including salmonids, are better protected against hypoxia than most mammals. Since hypoxic conditions are common for water-breathers, it is assumed that catecholamine induced inhibition of lipolysis is a strategy by which fishes are able to prevent accumulation of amphipatic molecules and hence membrane damage. In fish we observed low lipolytic rates combined with inhibiting effect of catecholamines on lipolysis in adipose tissue. The transduction mechanism of inhibition appears to be cAMP dependent and mediated via activation of β1- and possibly also β3- adrenoceptors. From in-vivo measurements in carp it appears that β2- adrenoceptor activation results in activation of lipolysis in the liver. Activation of lipolyis in trout liver and inhibition of lipolysis in tilapia adipocytes via β-adrenoceptors has been described. These opposite effects may explain the variable results obtained in the past. Further research focused on the expression of HSL in the different fat depots and the hormone sensitivity of these depots for lipolysis should be the next step in resolving the problems related to the regulation of lipolysis in fish. Figure 5. Model showing the action of α2- and β-adrenoceptors on the membrane bound adenylate cyclase. The α2-adrenoceptors bind to inhibiting G-proteins and the β-adrenoceptors to stimulating G-proteins. The interaction between the two adrenoceptors determines the activity of TG-lipase.