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Changes in Hormone Action during Aging: Glucocorticoid Regulation of Adipocyte Glucose Metabolism and Catecholamine Regulation of Myocardial Contractility (40956)

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Abstract. Many responses to hormones are altered during aging. Almost all the cellular components involved in mediating hormone actions have, in one situation or another, been implicated in such responsiveness changes. Glucocorticoid inhibition of glucose oxidation in rat adipocytes and catecholamine stimulation of rat heart contractile performance are two examples of reduced hormonal responsiveness during aging. In the former system, age changes have been reported in glucocorticoid receptor concentrations, rates of putative receptor biosynthesis, regulation of the glucose transport system, and the sulfhydryl content of the plasma membrane. In the latter system, no changes have been observed in catecholamine receptors, cyclic AMP production, or protein kinase activation. Instead, the age-associated functional change appears to be related to control of calcium entry into the cell. The manifestations of aging at the level of hormone action, therefore, appear to be multifaceted.

Some of the most important functional changes occurring during aging involve alterations in hormone action (1, 2). Many hormonal responses are not elicited to the same extent or exhibit different sensitivity profiles as organisms age. In an effort to elucidate the mechanisms by which such alterations in hormone action take place, many studies have focused on those cellular, molecular, and neuroendocrine systemic events which mediate hormonal responses. There is now evidence to suggest that aging changes may occur at all these levels. For example, hormone-responsive cells may be lost from certain tissues during aging, as in the case of ovarian corpora lutea and follicular cells (3). In other cases, neuroendocrine factors which are required for optimal cellular function may be deficient. This appears to be true in the case of rat uterine estrogen receptors which decrease concomitantly with circulating estrogen levels (4, 5) and rat prostate androgen receptors and responsiveness which are under positive androgenic control (6, 7).

Most investigations of age-altered hormone action have focused on molecular components and events, however. These include hormone binding to receptors, activation of adenylate cyclase and cyclic nucleotide metabolism, cell membrane function, receptor translocation, nuclear function, and activation of chromatin. Age changes have been reported in all of these categories (1, 2). Probably the most attention, however, has been paid to hormone receptors. Over 100 different receptor systems have been studied as a function of age during the adult life span ((1, 2) and review manuscript in preparation). About 70% of those receptors studied exhibit negative changes during part or all of adulthood and senescence. The reductions are mostly in apparent concentrations although a few examples of reduced binding affinities have also been described. Another 25% of the hormone receptors examined show no changes during aging, while about 5% actually seem to increase with age.

A number of decreases in receptor concentrations have been closely correlated with reduced responsiveness during aging. In other cases, receptors may decrease, but not to the same extent as particular hormonal responses. Certain receptors may be completely unchanged even though biological responses which they mediate may be substantially altered with age.

The object of the present manuscript is to review studies from this laboratory on two

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hormonal response systems which exhibit reduced capacity with increased age. These are glucocorticoid regulation of glucose metabolism in the rat epididymal fat pad adipocyte, and catecholamine regulation of contractile performance in the rat heart.

Glucocorticoid action during adipocyte aging. Adipocytes from rat epididymal fat pads provide a good model in which to study hormone action during postmitotic cellular aging. Isolation by collagenase digestion is simple, rapid, and yields a reasonably homogenous population of cells. Cell numbers are relatively static over the bulk of the adult rat life span (8). Thus, one is reasonably confident of dealing with the same cells in senescent and mature adult animals. Age differences observed by making such comparisons would not be due to shifts in hetereogeneous cell populations or loss of particular cell types during aging. Adipocytes are also highly active metabolically and responsive to many different hormones.

Glucocorticoids appear to be one of the more important classes of adipocyte regulatory hormones, with respect to both direct modulation of cellular energy metabolism and so-called indirect or permissive effects (9). When isolated rat epididymal fat pad adipocytes are exposed to dexamethasone for 2 hr, glucose oxidation is inhibited. The extent of this inhibition is about 50% in cells of young animals but is progressively reduced with increasing age (Table 1) (10). Dose–response curve shapes are not changed during aging, with 10^{-7} *M* hormone eliciting maximal response at all ages tested. Moreover, absolute basal levels of glucose oxidation do not change with age. Thus, age differences are comparable if expressed as percentages or in terms of picomoles of glucose metabolized (10).

The initial event in the elicitation of this response is the binding of glucocorticoid hormones to their cytoplasmic receptors (11). If receptors are occupied by other inactive steroids, addition of glucocorticoids has no effect on glucose metabolism. Due to the close relationship between receptor binding and responsiveness, receptor concentrations were assessed in adipocytes obtained from rats of different ages. As is the dose for maximal inhibition of glucose oxidation, glucocorticoid receptor levels are progressively reduced with increasing age (Table I) (10). Although the figures reported here are expressed per cell, since protein content in adipocytes is unchanged with age (10), similar reductions exist if receptor concentrations are expressed per milligram of protein.

Two possible explanations for age-related loss of glucocorticoid receptors and responsiveness are increased receptor degradation or decreased receptor synthesis in aged cells. In order to test the latter possibility a system was devised in which to measure the biosynthetic rate of "putative" glucocorticoid receptors (12). This

TABLE I. EFFECT OF AGE ON RAT ADIPOCYTE COMPONENTS AND EVENTS WHICH MEDIATE GLUCOCORTICOID ACTION[a]

	2–4 Months	10–13 Months	23–26 Months	Reference
Maximal inhibition of glucose oxidation by dexamethasone (%)	50 ± 8	21 ± 7	5 ± 2	(10)
Glucocorticoid receptor content per cell (number of binding sites)	56 ± 5 × 10^3	34 ± 5 × 10^3	14 ± 4 × 10^3	(10)
Putative glucocorticoid receptor biosynthetic rate (fmole of receptor/mg protein/hr)	–	1.05 ± 0.06	0.51 ± 0.07	(13)
Maximal inhibition of glucose transport by dexamethasone (%)	42 ± 2	−2 ± 5	2 ± 5	(16)
% of plasma membrane S groups in SH form	26 ± 4	22 ± 4	10 ± 2	(17)

[a] Values represent mean ± SE for four or more separate experiments.
[b] Assuming radioactive amino acid mix is not diluted by intracellular amino acid pools and random amino acid content of receptor.

term refers to a protein or proteins isolated by affinity chromatography on dexamethasone Sepharose which possess physiochemical properties essentially identical to those of native receptors in crude cytoplasmic preparations. Such properties include sedimentation coefficients of about 4 S on sucrose gradients at high ionic strength, molecular weights of about 50,000 on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, competition of binding by unlabeled dexamethasone, and thermal instability (12). Putative receptors were labeled by exposing isolated adipocytes to radioactive amino acids for varying periods of time. Labeling rates were then calculated in mature and senescent cells. Table I shows that the rate of putative glucocorticoid receptor biosynthesis in old cells is less than half that for mature adipocytes (13). Total protein synthesis, as evidenced by radioactive label in trichloroacetic acid-precipitable material, is not different between the two age groups, however (13). Thus, apparent differences in biosynthetic rates are probably not due to age differences in endogenous pools of amino acids or tRNAs and may be restricted to the products of a relatively few important regulatory genes (14, 15).

In addition to possible changes in glucocorticoid receptor metabolism, age-related reductions in responsiveness to these hormones may be due in part to generalized alterations in the cell membrane. This conclusion was reached following studies attempting to relate dexamethasone inhibition of glucose transport to inhibition of overall glucose oxidation (16). Table I shows that maximal glucocorticoid reduction of glucose transport and glucose oxidation are both 40–50% in adipocytes of 2- to 3-month-old rats. However, by 12 months of age, the ability to regulate glucose transport is lost, while dexamethasone inhibition of glucose oxidation is maintained at a reduced level. Failure to regulate transport in adipocytes of rats 12 months and older is also observed for the nonmetabolizable analogs 2-deoxy- and 3-*O*-methyl glucose. Subsequent experiments revealed that dexamethasone does inhibit glucose phosphorylation in older adipocytes, and thus overall glucose oxidation is still regulated (16). That the cell membrane becomes generally more refractory to regulation by both inhibitory and stimulatory agents was suggested by the fact that insulin, vitamin K5, and hydrogen peroxide are all essentially ineffective in stimulating glucose transport in older cells, even though two- to sixfold increases are obtained in young cells (16).

In order to obtain some insight into the physiochemical changes in the cell membrane which might be related to functional alterations during aging further studies were conducted. Since many investigators feel that aging may be related to oxidative damage, and since glucose transport function appears dependent upon the oxidation state of membrane protein sulfur groups, oxidized and reduced sulfur groups on the cell surface were quantitated as a function of age. A tritiated organic mercury–dextran probe was synthesized for this purpose (17). By analyzing the binding of this probe to intact epididymal fat pad adipocytes of rats of different ages it was determined that total membrane sulfur groups increased slightly as the animals aged but that the ratio of SH to total S groups was reduced by a factor of 2 to 3 between 2 and 24 months (Table I) (17).

Catecholamine action during myocardial aging. Like the adipocyte system discussed above, the heart is largely composed of fixed, postmitotic myocardial cells. The tissue is also responsive to many types of hormones and shows many functional changes during aging (18, 19).

The maximal ability of catecholamines to increase the rate of force development in isolated rat cardiac muscle has been shown to be decreased during aging (Table II) (20). These hormones are believed to stimulate contraction by initially interacting with β-adrenergic receptors on the cell membrane. Adenylate cyclase is then activated to produce cyclic AMP. Increases in cyclic AMP levels lead to increased activation of protein kinase, which in turn phosphorylates contractile proteins. Ultimately, the influx of calcium is stimulated and through calcium interaction with contractile proteins, contraction is enhanced.

TABLE II. EFFECT OF AGE ON RAT MYOCARDIAL COMPONENTS AND EVENTS WHICH MEDIATE CATECHOLAMINE ACTION[a]

	6–12 months	22–24 months	Reference
Maximal stimulation of rate of force development by isoproterenol (% of control)	210 ± 20	160 ± 20	(20)
β-adrenergic receptor concentration (fmole/mg membrane protein)	34.8 ± 1.5	39.0 ± 5.3	(20)
5×10^{-7} *M* isoproterenol stimulation of cAMP (pmole/mg wet wt)	0.617 ± 0.03	0.635 ± 0.01	(20)
5×10^{-7} *M* isoproterenol stimulation of protein kinase activation ratio (relative to maximum at 2 μM cAMP)	0.439 ± 0.02	0.434 ± 0.02	(20)
Stimulation of rate of force development at 1 m*M* Ca^{2+} (% of control at 0.3 m*M* Ca^{2+})	245 ± 15	270 ± 18	(20)

[a] Values represent mean ± SE for four or more separate experiments.

An experimental rationale similar to that used to elucidate the mechanisms of altered glucocorticoid responsiveness in aged adipocytes was applied to the heart catecholamine-contraction system. Since receptors are the initial sites of hormone action, β-adrenergic receptors were measured in hearts of mature and senescent rats. Table II shows that unlike the age-related reduction seen for adipocyte glucocorticoid receptors, no change in β-adrenergic receptor concentrations is observed during aging (20). Similarly, no change in receptor affinity could be demonstrated.

Table II also shows that isoproterenol stimulation of cyclic AMP and protein kinase activation are comparable in both mature and senescent hearts (20). These data coupled with the fact that receptor concentration and affinity are not changed with age suggest that the cause of altered contractile responsiveness lies further along in the scheme.

Since catecholamine stimulation of contraction is ultimately mediated by increased calcium delivery to contractile proteins, the effect of raising the calcium concentration was examined. When this is increased from 0.3 to 1 m*M* both mature and senescent hearts exhibit the same stimulated contractile response (Table II) (20). Thus it appears that the age-related change occurs at a step prior to calcium influx into the myocardium.

Conclusions. A number of age-related changes in the events which mediate hormone actions have been described in the literature (1, 2, 10, 13, 16, 17, 20). Two hormonal response systems, representative of the types of age changes which occur, have been reviewed here. As with many hormonal responses, both glucocorticoid inhibition of glucose oxidation in rat adipocytes and catecholamine stimulation of rat cardiac contraction show decreased maxima with increasing age (10, 20).

In the case of glucocortocoid action, the decreased responsiveness appears to be at least partially due to a loss of glucocorticoid receptors which may be the result of a decreased biosynthetic rate (10, 13). On the other hand, no changes in catecholamine β-adrenergic receptors during aging are observed in the rat heart (20).

Events subsequent to β-adrenergic receptor activation, such as elevation of cyclic AMP levels and stimulation of protein kinase, also appear not to change in senescent rat hearts (20). Instead, the alteration most closely related to decreased contractile responsiveness seems to somehow involve the stimulation of calcium entry into the cell, since at elevated calcium concentrations mature and senescent hearts perform equally well (20).

Components of hormone action other than receptors also change in rat epididymal fat pad adipocytes during aging and may be involved in altered glucocorticoid responsiveness. The cell membrane transport system for glucose appears to become generally more refractory to regulation by both stimulatory and inhibitory agents (16).

These include insulin, vitamin K5, and hydrogen peroxide, which increase the rate of glucose transport and dexamethasone which retards it. In addition, cell surface sulfhydryl groups become progressively more oxidized as age increases (17). Such chemical changes may also play a role in the age-related functional impairments already described.

It is therefore important to examine various components involved in hormone action in order to elucidate the mechanisms by which age changes in responsiveness occur. Even in cases where receptor changes correlate closely with altered responsiveness, other events mediating hormonal actions may also be affected. Manifestations of aging may thus occur at many levels of hormone action and by studying changes at all these levels a more fundamental understanding of the basic mechanisms of aging may be attained.

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