review

Adrenergic regulation of adipocyte metabolism

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Abstract Adipocytes can be readily isolated from intact adipose tissue. In adipocytes from hamster and human white adipose tissue it is possible to demonstrate beta, alpha$_1$, and alpha$_2$ adrenoceptors. Alpha$_2$ adrenoceptor activation inhibits while beta adrenoceptor activation stimulates cyclic AMP accumulation and lipolysis. The effects of catecholamines on cyclic AMP accumulation are mediated through regulation of adenylate cyclase activity, which is activated through beta adrenoceptors and inhibited through alpha$_2$ adrenoceptors. Activation of alpha$_1$ adrenergic receptors has been shown to be associated with elevations of cytosol calcium and increased turnover of phosphatidylinositol. In white adipocytes, the only known alpha$_1$ adrenergic effects are inhibition of glycogen synthase and stimulation of glycogen phosphorylase via mechanisms distinct from those by which cyclic AMP produces similar end effects. In brown adipocytes, alpha$_1$ adrenoceptor activation stimulates respiration. Thyroid hormones primarily regulate the sensitivity of adipocytes to beta-adrenergic amines while having little effect on alpha adrenoceptor sensitivity.—**Fain, J. N., and J. A. García-Sáinz.** Adrenergic regulation of adipocyte metabolism. *J. Lipid Res.* 1983. **24:** 945–966.

I. Separation of catecholamine effects into beta$_1$, beta$_2$, alpha$_1$, and alpha$_2$

Probably at least four classes of receptors exist for catecholamines. Ahlquist (1) originally divided the effects of catecholamines into two groups which he called alpha and beta. Subsequently it was found that the beta effects of catecholamines are generally associated with increases in cyclic AMP (2). Lands et al. (3) subdivided the beta effects of catecholamines into beta$_1$ and beta$_2$ based on structure-activity relationships for agonists. The beta$_2$ adrenoceptors are involved in bronchodilation and are preferentially activated by epinephrine, while norepinephrine has little activity. In contrast, norepinephrine and epinephrine are equipotent activators of beta$_1$ adrenoceptors in heart and adipose tissue (4). Compounds with bulky groups (tertiary butyl) on the amine carbon such as terbutaline and albuterol are preferential beta$_2$ agonists, while a compound such as prenalterol is more active at beta$_1$ sites (4). Ariens et al. (5) suggested that the beta$_1$ effects are due to norepinephrine released by sympathetic nerves, while beta$_2$ effects are due to hormonal effects of epinephrine released by the adrenal medulla (**Fig. 1**).

Alpha effects of catecholamines are also divided into alpha$_1$ and alpha$_2$ types (6–9). There appear to be separate receptors for the alpha$_1$ versus the alpha$_2$ effects (9), which is hardly surprising since they have different biochemical actions. Wikberg (7) and Fain and García-Sáinz (8) suggested that alpha$_1$ effects involve turnover of phosphatidylinositol and elevation of cytosol Ca^{2+}, while alpha$_2$ effects involve inhibition of adenylate cyclase. However, in cells in which the level of cyclic AMP affects the cytosol Ca^{2+} pool or the biochemical actions of Ca^{2+}, there will be effects of alpha$_2$ activation on Ca^{2+} action (**Table 1**). These cells are subject to what might best be called antagonistic control since Ca^{2+} and cyclic AMP have opposite effects (10). Smooth muscle is a

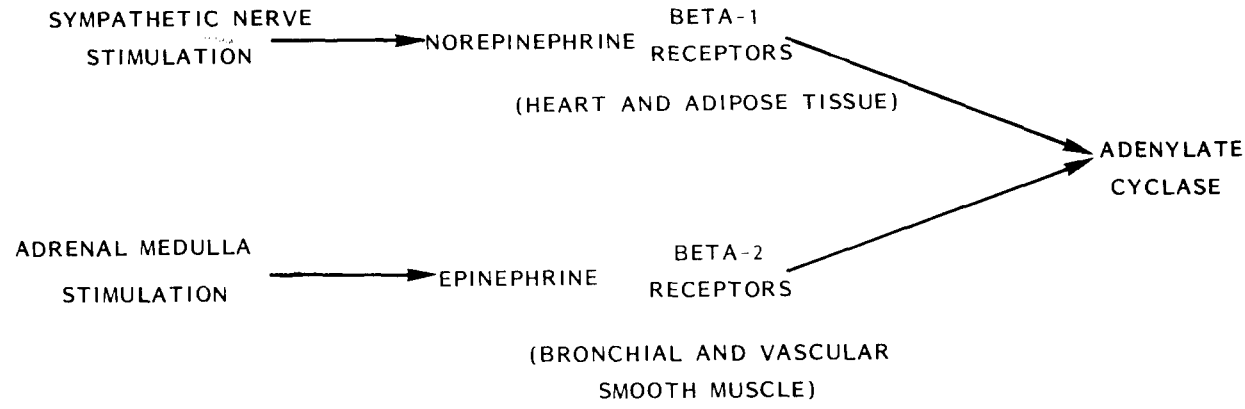

Fig. 1. Differentiation of $beta_1$ and $beta_2$ adrenergic responses.

good example of this since elevation of Ca^{2+} by $alpha_1$ receptors or lowering of cyclic AMP by $alpha_2$ receptors both produce contraction while activation of beta receptors produces relaxation. However, in cells exhibiting coordinate control (10), cyclic AMP and Ca^{2+} have similar effects. In these cells $alpha_1$ adrenoceptor activation elevates Ca^{2+} and this has the same result as elevation of cyclic AMP (Table 1).

McGrath (11) suggested that more than one type of $alpha_1$ receptor might exist. Furthermore, he suggested that $alpha_1$ responses are due to sympathetic release of norepinephrine at nerve endings. In contrast, epinephrine released as a hormone by the adrenal medulla works more slowly and mediates $alpha_2$ responses (**Fig. 2**).

$Alpha_1$ catecholamine action appears to involve elevation of cytosol Ca^{2+} and in every tissue in which $alpha_1$ catecholamine effects can be demonstrated there is an increase in $[^{32}P]P_i$ uptake into phosphatidylinositol (8, 12). The meaning of this is unknown but is discussed in the section on $alpha_1$ catecholamine action.

A correlation also exists between inhibition of adenylate cyclase and $alpha_2$ catecholamine effects. In platelets (13) and rat pancreatic islets (14), it has been possible to demonstrate a reduction of basal cyclic AMP values under some but not all conditions after addition of $alpha_2$ catecholamines. The poor correlation between total cyclic AMP and metabolic responses has prompted interest in the question of whether $alpha_2$ and beta catecholamines regulate other enzymes besides adenylate cyclase.

The four types of adrenoceptors are listed in **Table 2.** The natural agonist for each along with the most selective agonists and antagonists available as well as the known intracellular effects are shown in Table 2.

There has been much progress in our understanding of the nature of the alpha and beta catecholamine effects on adipocytes over the past few years. Brief reviews on this subject have been published by Lafontan and Berlan (15) and by García-Sáinz and Fain (16). Fain (17, 18) has also reviewed in detail the hormonal regulation of lipolysis by hormones with emphasis on the role of cyclic nucleotides, while Kather (19) has reviewed human adipocyte lipolysis with emphasis on pathogenesis of

TABLE 1. Relationship between Ca^{2+} and cyclic AMP as intracellular messengers

	Tissue	$Alpha_1$ Response	$Alpha_2$ Response	Effect of Cyclic AMP
Antagonistic control	Smooth muscle	Contraction	Contraction	Relaxation
	Platelets	None	Aggregation	Inhibition of aggregation
Coordinate control	Endocrine pancreas	None known	Lower insulin release	Increase insulin release
	Liver	Increase glycogen phosphorylase		Increase glycogen phosphorylase
	Adipocytes	Inactivate glycogen synthase	Activate glycogen synthase	Inactivate glycogen synthase
		Little effect on lipolysis	Inhibit lipolysis	Activate lipolysis

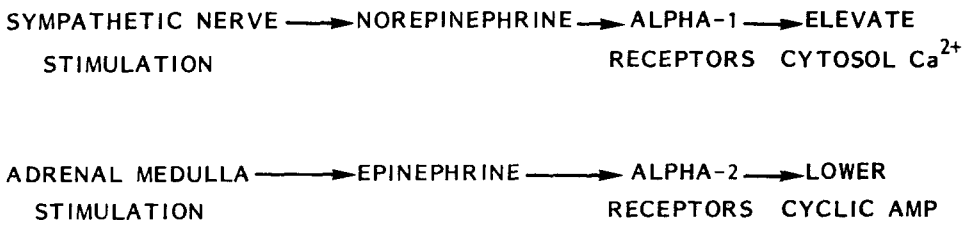

Fig. 2. Differentiation of $alpha_1$ and $alpha_2$ adrenergic responses.

obesity. This review covers recent advances in the adrenergic regulation of adipocyte metabolism.

II. Activation of lipolysis via beta adrenoceptors

Activation of beta adrenoceptors in adipocytes is associated with stimulation of adenylate cyclase, protein kinase, and triacylglycerol lipase (17–21). Fain (20) suggested 10 years ago that the adrenoceptors in rat adipocytes were of the $beta_1$ subtype. This was based on four findings. *1,* Norepinephrine is more potent than epinephrine as an activator of lipolysis. *2,* Isoproterenol is 5-fold more potent than norepinephrine. *3,* A compound such as albuterol [alpha-1(t-butylaminomethyl)-4-hydroxy-m-xylene-$alpha_1$, $alpha_2$-diol] is much less active than isoproterenol as an activator of lipolysis. *4,* Butoxamine, which is a $beta_2$ antagonist, is relatively ineffective as an inhibitor of lipolysis.

Further studies have confirmed this general scheme while indicating that there are subtle differences between the $beta_1$ adrenoceptors of heart and adipose tissue. Antagonists and agonists do not have the same effects on adipose tissue as on heart (22–24). However, there is no evidence for a mixed population of $beta_1$ and $beta_2$ adrenoceptors in adipose tissue (4, 19, 22–24). It has been suggested that the rat adipocyte receptor has a dualistic or hybrid character, somewhat between the $beta_1$ adrenoceptors of the heart and the $beta_2$ adrenoceptors of the trachea (22–24). This is based on the fact that practolol is considerably less effective than propranolol as an antagonist of $beta_1$ adrenoceptor responses in human adipocytes as opposed to the heart (25). Thus, beta adrenergic antagonists that affect cardiac response have much less effect on human adipocyte lipolysis. However, $beta_2$ selective agonists have so little effect on adenylate cyclase activity of human adipocyte ghosts (25) that, from the agonist perspective, the receptors are rather like the $beta_1$ receptors present in the heart.

Jacobsson et al. (26) recently suggested that two subpopulations of $beta_1$ adrenoceptors exist in human adipocytes. They found that prolonged incubation of human adipocytes with norepinephrine decreased by 50% the binding sites for dihydroalprenolol. Furthermore, in membranes exposed to catecholamines and 1 mM N-ethylmaleimide for 10 min, approximately 50% of the binding sites disappeared, while there was no loss due to exposure to N-ethylmaleimide alone and 14% in response to isoproterenol (26). Another interpretation of the data is that only half of the binding sites for the catecholamine antagonist dihydroalprenolol are true $beta_1$ adrenoceptors. Whatever the explanation, it is clear that agonist binding to human adipocyte membranes markedly alters the ability of half the sites to bind catecholamine antagonists.

The work of Lai, Rosen, and Rubin (27) indicates that the beta adrenoceptors of the cultured 3T3-L1 fibroblasts, which have differentiated into cells that accumulate triglyceride and are adipocyte-like in appearance, are $beta_2$ adrenoceptors. In contrast, the receptors of preadipocytes (3T3-L1 fibroblasts) are $beta_1$ in character and apparently not linked to any metabolic response. The switch in type of beta adrenoceptors (based on binding studies with iodohydroxybenzylpindolol) was brought about by addition of dexamethasone, a synthetic glucocorticoid (27). Isoproterenol was able to activate adenylate cyclase in membranes from control 3T3 adipocytes, but the log-dose response curve was shifted to the left by one order of magnitude after dexamethasone exposure. However, the maximal activity was unaltered (27).

TABLE 2. Four types of catecholamine effects

Type of Adrenoceptor	Natural Agonist	Synthetic Agonist	Specific Inhibitors	Intracellular Effect
$Beta_1$	Norepinephrine	Prenalterol	Metoprolol	Increases cyclic AMP
$Beta_2$	Epinephrine	Terbutaline	Butoxamine	Increases cyclic AMP
$Alpha_1$	Norepinephrine	Phenylephrine or amidephrine	Prazosin	Increases cytosol Ca^{2+}
$Alpha_2$	Epinephrine	Clonidine	Yohimbine	Lowers cyclic AMP

The results of Lai et al. (27) suggest that activation of either $beta_1$ or $beta_2$ receptors is associated with activation of adenylate cyclase (2, 4). Possibly $beta_1$ adrenoceptor activation is associated with something other than adenylate cyclase activation (28, 29). This was based on the finding that, in incubated pieces of rat parotid or guinea pig submandibular glands, the addition of terbutaline (a specific $beta_2$ agonist) markedly stimulated cyclic AMP accumulation while having only a small effect on amylase release. In contrast, prenalterol, a specific $beta_1$ agonist, markedly stimulated amylase release while having less effect on cyclic AMP accumulation than terbutaline. However, this could be due to a stimulation of cyclic AMP accumulation by terbutaline in cells other than those involved in release of amylase. A similar problem in adipose tissue with regard to prostaglandins of the E series, stimulating cyclic AMP accumulation while inhibiting lipolysis, was resolved by the discovery that prostaglandins stimulated adenylate cyclase activity in the endothelial cells of the extensive capillary bed while inhibiting that in adipocytes (30). In isolated rat adipocytes, which are a relatively homogeneous population of a single cell-type, prostaglandins inhibit both lipolysis and adenylate cyclase (30).

In adipocytes isoproterenol elevates both cyclic AMP and lipolysis. However, a low concentration of isoproterenol can elevate lipolysis while having no detectable effect on total cyclic AMP (31). In contrast, with forskolin, a diterpene that activates adenylate cyclase in a wide variety of mammalian cells (32), activation of lipolysis is only seen if there is an increase in total cyclic AMP (31). Similar results have been seen in isolated rat adrenal cells. Forskolin stimulation of steroidogenesis was associated with increase in total cyclic AMP while ACTH stimulated steroidogenesis at concentrations that had no effect on total cyclic AMP (33). In adrenal cells and adipocytes, a low concentration of forskolin potentiated the effects of the target hormones on both cyclic AMP and the physiological response (31, 33). These results could be explained by hormonal activation of another enzyme in addition to adenylate cyclase, or by postulating that hormones preferentially increase cyclic AMP in a compartment where the triacylglycerol lipase is localized.

Possibly, the receptor subunit of cyclic AMP-dependent protein kinase is loosely bound to the hormone receptor as a peripheral protein. Thus, when the hormone receptor complex activates adenylate cyclase, there is a high local concentration of inactive cyclic AMP-dependent protein kinase. The cyclic AMP binds to the regulatory subunit of this kinase, which results in release of the catalytic subunit that diffuses through the cytosol to activate triacylglycerol lipase. In contrast, when adenylate cyclase is activated by forskolin, there are fewer protein kinases around as it bypasses the hormone receptors. However, when cyclic AMP increases sufficiently, the soluble cyclic AMP-dependent protein kinase is activated and the triacylgycerol lipase is phosphorylated. This hypothesis would not require any other effect of beta catecholamines besides activation of adenylate cyclase.

Alternatively, beta-adrenoceptor activation could activate some other process. Fain (17) suggested that activation of substrate for lipolysis through a cyclic AMP-independent mechanism might be involved in hormonal activation of lipolysis. If this occurs it is likely to be through a process that potentiates the action of cyclic AMP, since forskolin activates lipolysis and potentiates catecholamine action (31).

III. $Beta_1$ vs. $beta_2$ catecholamine effects on adipocyte cyclic AMP accumulation and lipolysis

In studies with rat adipocytes, no evidence was found that a specific $beta_1$ agonist such as prenalterol did anything other than elevate cyclic AMP (**Fig. 3**). Rat adipocytes were incubated with various concentrations of prenalterol or terbutaline either without or with adenosine deaminase plus theophylline. Lipolysis was measured after 60 min and cyclic AMP at 5 min. Prenalterol was a less effective stimulus of both lipolysis and cyclic AMP accumulation than was terbutaline, but the relationship between the two parameters was the same in the presence of prenalterol as with terbutaline.

The results in Fig. 3 also demonstrate the typical correlation seen between measurements of total cyclic AMP and lipolysis in adipocytes (34–36). Cyclic AMP values between 25 and 35 pmol/10^6 cells were associated with no detectable lipolysis up to half-maximal activation of lipolysis. Cyclic AMP values in excess of 50 pmol/10^6 cells or more were associated with maximal activation of lipolysis. Cyclic AMP values as high as 5000 pmol/10^6 cells were seen but no further activation of lipolysis was seen. These data suggest that cyclic AMP is related to lipolysis but that it is very difficult to accurately measure the small increases that regulate lipolysis.

The effects of selective beta agonists on adenylate cyclase are shown in **Fig. 4.** Prenalterol activated adenylate cyclase but less effectively than terbutaline. Terbutaline in turn was considerably less potent than isoproterenol as an activator of adenylate cyclase (Fig. 4), which is expected if rat adipose tissue has typical $beta_1$ adrenoceptors. We conclude from these data that, in rat adipocytes, there is no evidence that $beta_1$ agonists have any different effects on adenylate cyclase activation or lipolysis than $beta_2$ agonists.

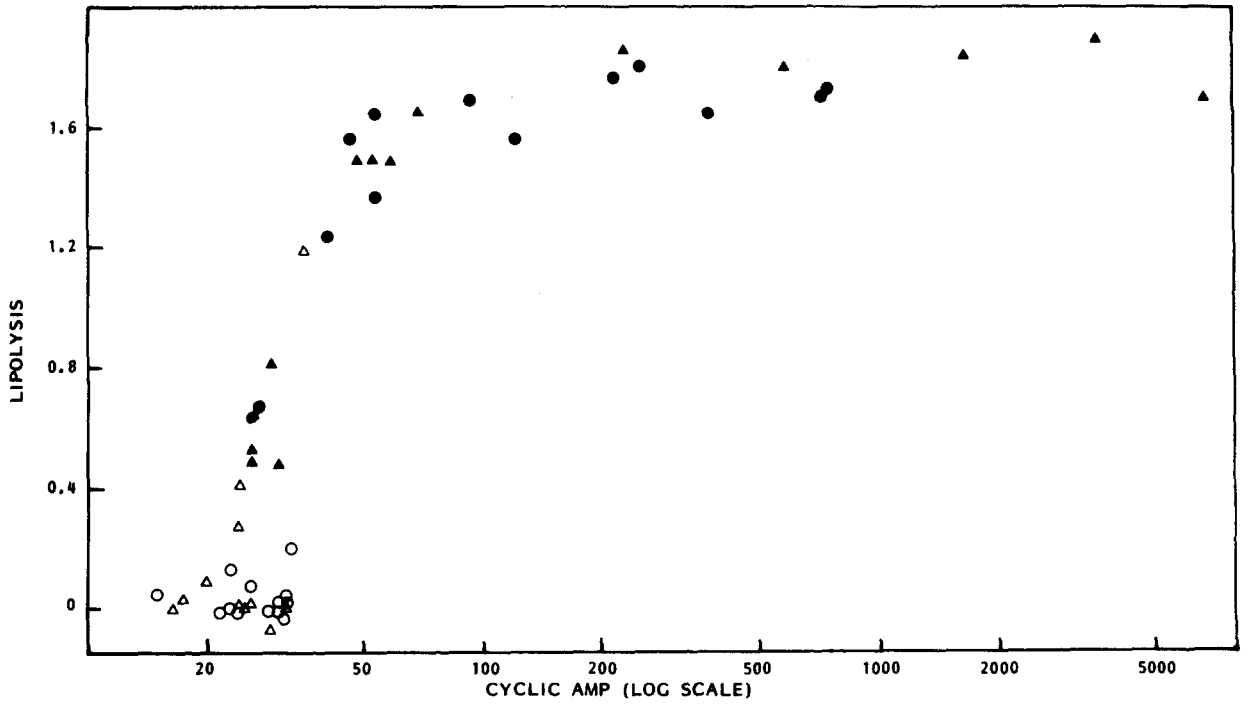

Fig. 3. Correlation between total cyclic AMP and lipolysis in rat adipocytes. Isolated rat adipocytes (2×10^5) were incubated for 5 min (cyclic AMP determinations) or 60 min (lipolysis) in 1 ml of medium containing 3% albumin and varying concentrations (10^{-9} to 10^{-4} M) of prenalterol (circles) or terbutaline (triangles). The open symbols are for cells incubated without and the solid symbols are for those incubated with 1 μg/ml of adenosine deaminase and 100 μM theophylline. Cyclic AMP is in pmol/10^6 cells and lipolysis (glycerol release) is in μmol/10^6 cells. Cyclic AMP was also measured after 60 min incubation and the maximal values were 13–20% of those seen at 5 min, but in every case in which cyclic AMP was elevated over basal at 5 min, it was similarly elevated at 60 min. The values for each point are the means of two paired experiments (unpublished studies, J. Fain).

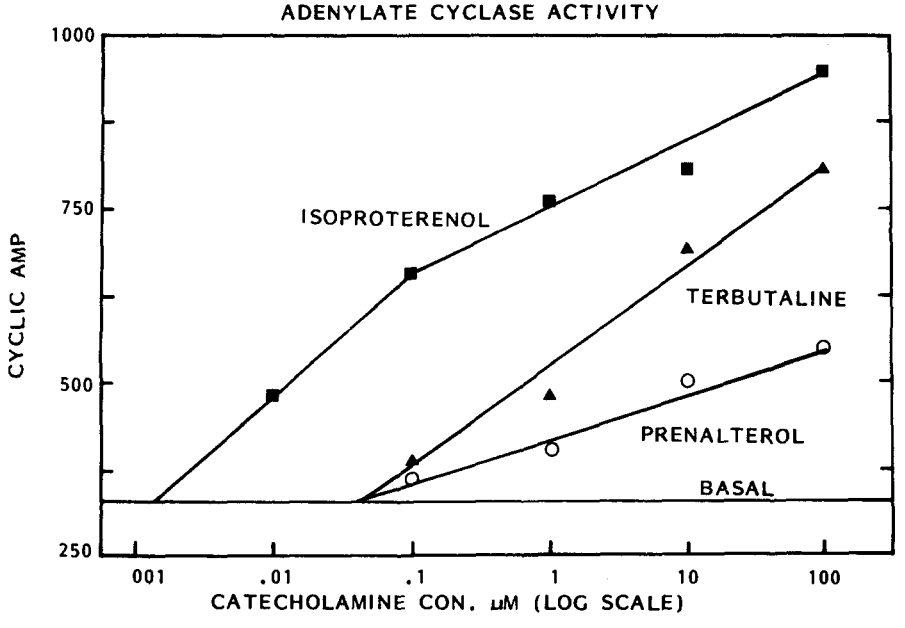

Fig. 4. Activation of rat adipocyte plasma membrane adenylate cyclase activity by isoproterenol, prenalterol, and terbutaline. Isolated rat adipocytes were homogenized in isotonic sucrose containing 10 mM Tris (pH 7.4) and 1 mM EDTA. The precipitate obtained after centrifugation at 15,000 *g* for 15 min was resuspended in isotonic sucrose and layered over 32% sucrose. The interface material obtained after centrifugation was used as the plasma membrane fraction. Adenylate cyclase activity was assayed in the presence of 1 μM Gpp (NH)p over a 20-min incubation at 30°C. Cyclic AMP accumulation is expressed as pmoles of cyclic AMP formed per mg of protein over 1 min and the values are the means of three paired experiments. Basal activity of adenylate cyclase was 330 and is shown by the line marked basal (unpublished studies, J. Fain and C. O'Donnell).

Adipocyte lipolysis appears to correlate with phosphorylation of the hormone-sensitive lipase. Belfrage et al. (21) have reviewed the evidence for the presence of a triacylglycerol lipase in adipose tissue that is directly activated by phosphorylation via a cyclic AMP-dependent protein kinase and inactivated via dephosphorylation due to a protein phosphatase. Corbin et al. (37) and Huttunen, Steinberg, and Mayer (38) first published in 1970 that rabbit muscle protein kinase in the presence of cyclic AMP and ATP enhanced triacylglycerol lipase activity. Recently the triacylglycerol lipase has been purified and found to have a M_r of around 84,000 (39).

Our laboratory engaged in a collaborative study with that of Belfrage to compare the effects of beta adrenoceptor activation on lipolysis, total cyclic AMP accumulation, and phosphorylation of the triacylglycerol lipase. A concentration of norepinephrine (0.1 μM), which gave almost 80% activation of lipolysis, increased ^{32}P uptake into the triacylglycerol lipase by almost 50% but had little effect on total cyclic AMP accumulation. Increasing the concentration of norepinephrine to 0.5 μM gave maximal activation of lipolysis and of ^{32}P uptake into lipase (120% increase) and transiently elevated cyclic AMP at 1 and 3 min. By 5 min, total cyclic AMP in the presence of 0.1 μM norepinephrine had returned to control values but the degree of phosphorylation of the enzyme was further elevated.[1]

These data suggest that while cyclic AMP may be the major factor regulating lipolysis, there is probably some compartmentation of cyclic AMP in adipocytes as mentioned earlier in this section. We conclude that no good evidence exists that disproves the hypothesis that $beta_1$ and $beta_2$ effects of catecholamines are secondary to elevation of adenylate cyclase although other mediators might be involved.

IV. Inhibition of cyclic AMP accumulation and lipolysis through $alpha_2$ adrenoceptors

A. General aspects. About 15 years ago Turtle and Kipnis (40) suggested that alpha-adrenergic effects were mediated through inhibition of adenylate cyclase. However, in many tissues alpha-adrenergic activation did not decrease cyclic AMP accumulation. Furthermore, under some circumstances alpha-adrenergic activation actually increased production of cyclic AMP. The general confusion about the effects of alpha-adrenergic amines on adenylate cyclase was clearly expressed in a review in 1980 in which the author stated: "In summary, it appears that alpha agonists can inhibit, stimulate, or be without effect on adenylate cyclase, depending on the tissue or condition" (41).

[1] Belfrage, P., and J. N. Fain. Unpublished studies.

The availability of selective agonists and antagonists for $alpha_1$ and $alpha_2$ adrenoceptors has aided our understanding of which alpha effects are involved in inhibition of adenylate cyclase. Furthermore, some increases in cyclic AMP due to alpha-adrenergic activation are secondary effects due to prostaglandin generation mediated through $alpha_1$ adrenoceptors (42, 43).

Sabol and Nirenberg (44) first demonstrated that the inhibition of adenylate cyclase by catecholamines in membrane preparations derived from neuroblastoma-glioma cells was mediated through $alpha_2$ adrenoceptors. It has been suggested that all $alpha_2$ catecholamine effects are due to inhibition of adenylate cylase (7, 8). Kato and Nakaki (45) suggested that the $alpha_2$ adrenergic inhibition of insulin release was exerted at a step distal to cyclic AMP generation. However, this conclusion was based on the assumption that if dibutyryl cyclic AMP-induced insulin release was inhibited by clonidine, this indicated an affect elsewhere than on adenylate cyclase activation.

There are many cells in which inhibition of adenylate cyclase by $alpha_2$ catecholamines can be demonstrated (**Table 3**). It is likely that inhibition of insulin release and stimulation of platelet aggregation are due to inhibition of adenylate cyclase. Since basal cyclic AMP values are low in these cells it is difficult to demonstrate decreases in cyclic AMP if most of the cyclic AMP is bound and inactive. Therefore, there could be a 50% drop during $alpha_2$ adrenoceptor activation of the cyclic AMP pool involved in regulation of function but, if this pool is only a small fraction of total cyclic AMP, the decrease may be difficult to detect.

In rat pancreatic islets, Yamazaki, Katada, and Ui (56) found an excellent correlation between levels of cyclic AMP and insulin release due to $alpha_2$ adrenoceptor activation if all experiments were done in the presence of 0.5 mM isobutyl methylxanthine (IBMX) and 16.5 mM glucose. These are conditions in which cyclic AMP values are high and lowering cyclic AMP reduces insulin release. Rabinovitch, Cerasi, and Sharp (57) also found a good inhibition of insulin release and cyclic AMP if islets were stimulated with either glucagon or 0.1 mM IBMX and 3.3 mM glucose. However, they suggested that since it took more epinephrine in their hands to inhibit basal cyclic AMP formation than to inhibit insulin release, something else was involved besides cyclic AMP. They measured the accumulation of labeled cyclic AMP in cells where the ATP pool had previously been labeled by incubation with tritiated adenine. This may be more sensitive than measuring total cyclic AMP. The difficulty of correlating dose response curves for stimulation of cyclic AMP accumulation due to catecholamines has been discussed previously. It remains to be determined whether $alpha_2$-adrenergic ac-

TABLE 3. Cells showing alpha$_2$-adrenergic effects and relationship to inhibition of adenylate cyclase

Tissue	Inhibition of Adenylate Cyclase	Metabolic Effect and Relationship to Decrease in Cyclic AMP
Hamster adipocytes	Yes (46–48)	Inhibition of hormonal activation of lipolysis and of cyclic AMP (49–51)
Human adipocytes	Yes (52, 53)	Same as hamsters (53–55)
Pancreatic islets	Yes (56)	In the presence of methylxanthine and glucose, an excellent correlation is seen between inhibition of insulin release and cyclic AMP accumulation (40, 56). In the absence of methylxanthine, there is little detectable effect on basal cyclic AMP but insulin release is still inhibited by alpha$_2$ adrenoceptor activation (14, 56, 57).
Human platelets	Yes (58, 59)	Inhibition of basal cyclic AMP formation seen if platelets were prelabeled with adenosine and formation of radioactive cyclic AMP was examined (13). However, platelet aggregation readily seen even if total cyclic AMP was not decreased (13).

tivation has effects other than inhibition of adenylate cyclase, but this accounts for alpha$_2$ effects just as stimulation of adenylate cyclase accounts for beta effects.

B. Adenylate cyclase regulation in adipocytes. It is currently accepted that at least two molecular entities are involved in activation of adenylate cyclase by hormones, i.e., the receptor and a stimulatory guanine-nucleotide binding "N_s" protein in addition to the catalytic unit of the enzyme. Similarly, two entities seem to be involved in inhibition of adenylate cyclase by hormones, i.e., the receptor and an inhibitory guanine-nucleotide binding protein "N_i". Hormonal activation of adenylate cyclase involves binding of GTP at the stimulatory guanine-nucleotide regulatory site of the "N_s" protein and inactivation of the cyclase may be due to hydrolysis of GTP at the regulatory site by GTPase (60). Consistent with this, Jakobs and coworkers (48, 61) have shown that inhibition of adipocyte adenylate cyclase by hormonal factors is associated with acceleration of the turn-off reaction (48) and activation of the low K_m GTPase activity (61). GTP exerts a biphasic effect on adipocyte adenylate cyclase, i.e., activation is observed at low concentration whereas inhibition is produced at high concentrations (62). Mercurial treatment abolishes the activatory phase but not the inhibitory effects of GTP (62). In addition, studies using radiation inactivation suggest that structures of different size (63) are involved in inhibition and activation of fat cell adenylate cyclase by GTP.

High concentrations of sodium (100 mM) enhance the inhibition of adenylate cyclase by hormonal factors in hamster fat cell ghosts (46, 47, 64). Therefore, it has been suggested that a requirement for sodium may exist. However, inhibition of cyclic AMP accumulation by several hormonal factors can be clearly observed in hamster adipocytes incubated in medium without sodium (65). These data raise doubts about an absolute requirement for sodium in the medium.

Forskolin activates adenylate cyclase and increases lipolysis in rat (31) and human (66) adipocytes. The stimulatory effects of forskolin on lipolysis and cyclic AMP accumulation in human adipocytes were inhibited by alpha$_2$ adrenoceptor activation (66). Direct inhibition of forskolin-activated adenylate cyclase by alpha$_2$ adrenergic amines was also observed in human adipocyte ghosts (66). These results indicate that the alpha$_2$ adrenoceptor, after binding to the N_i protein, is able to inhibit adenylate cyclase activated by nonhormonal agents (forskolin).

C. Pertussis toxin inhibition of alpha$_2$ catecholamine inhibition of adenylate cyclase. Administration of pertussis vaccine to hamsters markedly diminishes the sensitivity of their adipocytes to hormonal factors that inhibit adenylate cyclase (67). It was suggested that the toxin also known as IAP (islet-activating protein) present in the vaccine blocks the inhibitory effects of alpha$_2$ adrenergic receptor activation on adenylate cyclase (67). Hazeki and Ui (68) and Katada and Ui (69) have obtained similar results using other experimental models. More recently, Katada and Ui (70, 71) found that pertussis toxin ADP-ribosylates a protein with a different molecular weight than the protein that is ADP-ribosylated by cholera toxin. It is thought that this is the so-called inhibitory guanine-nucleotide binding protein (N_i) involved in inhibition of adenylate cyclase by adenosine, alpha$_2$ catecholamines, and prostaglandins. These agents only affect adenylate cyclase activity in the presence of GTP (44, 46, 48, 62, 63). Recently, we found that the inhibitory effects of guanine nucleotides on adenylate cyclase activity of rat adipocyte membranes were abolished by pertussis toxin (72).

The administration of pertussis vaccine (which contains the toxin) to rats did not elevate their basal levels of plasma fatty acid or those seen 30 min after the injection of epinephrine (73). However, chopped epididymal fat pads from pertussis-treated rats had a high rate

of basal lipolysis that was blocked by beta adrenergic antagonists but not by prostaglandin E (74). The addition of norepinephrine to the chopped fat pads from normal rats gave about the same rate of lipolysis as that seen under basal conditions in fat pads and was blocked by prostaglandin E_1. We have seen a similar increase in basal lipolysis in adipocytes from pertussis toxin-treated rats which was accompanied by a 10- to 100-fold increase in the sensitivity of the cells to the stimulation of cyclic AMP accumulation and lipolysis by both norepinephrine and forskolin.[2] The adipocytes from toxin-treated rats had a normal response to insulin both with respect to inhibition of lipolysis and stimulation of glucose metabolism.[2] However, the inhibitory effects of N^6(phenylisopropyladenosine) were abolished by toxin treatment as noted previously in hamster adipocytes (67).

The tremendous enhancement of the lipolytic response to catecholamines in rat adipocytes by pertussis toxin suggests that the inhibitory guanine-nucleotide binding protein plays a very important role in the physiological regulation of lipolysis by hormones. Studies are in progress to examine the role of this protein in the response to thyroid hormones, glucocorticoid, and growth hormone, which all regulate lipolysis through unknown mechanisms (17, 18, 20). In rat adipocytes there are no alpha$_2$ adrenergic responses, which leaves adenosine, nicotinic acid, and prostaglandins as agents that work through the inhibitory guanine-nucleotide binding protein. The effects of pretreatment with pertussis toxin are remarkably similar to those that can be produced by the addition of adenosine deaminase to the medium (75). These data support the suggestions of Fain (76) and Schwabe, Ebert, and Erbler (77) that adenosine is important in the regulation of adipocyte lipolysis and cyclic AMP accumulation. There is no evidence as yet that hormones stimulate adenosine formation or release in adipocytes, rather there appears to be enough in isolated adipocytes to restrain hormonal activation of adenylate cyclase (78–80), which is supported by the pertussis toxin studies.

The administration of pertussis toxin to hamsters increased the basal levels of plasma free fatty acids by 3-fold and elevated liver triacylglycerols, plasma triacylglycerols, and ketone bodies (81). These are much greater effects than reported in rats and may be related to the fact that hamster adipocytes have inhibitory alpha$_2$ adrenergic receptors that are not present in rat adipocytes. This could be interpreted as suggesting that alpha$_2$ adrenoceptors are important in the regulation of lipolysis in hamsters. In contrast, in rats, inhibition of just the adenosine and prostaglandin response can be compensated for in the intact animal so that in vivo lipolysis is restrained. Whether this is the correct explanation or not, the studies with pertussis toxin have opened up new avenues for the exploration of adipocyte metabolism.

The effects of cholera and pertussis toxins are summarized in **Fig. 5.** Cholera toxin mimics the effects of beta adrenoceptor activation on adenylate cyclase. Pertussis toxin blocks alpha$_2$ adrenoceptor action as well as that of adenosine. Both toxins ADP-ribosylate proteins but not the same ones (70, 71). Both toxins also markedly increase activation of adenylate cyclase by GTP. However, only cholera toxin markedly increases basal adenylate cyclase activity (70, 71).

D. Desensitization. Prolonged activation of receptors that stimulate adenylate cyclase often leads to a decreased responsiveness of the target cell. This complex phenomenon is known as desensitization and in some cases is associated with loss of receptors. In hamster adipocytes exposed to an alpha$_2$-selective adrenergic agonist, such as clonidine, for periods up to 2 hr, no adrenergic desensitization was seen.[3] Burns and coworkers (82) recently reported that exposure of human fat cells to isoproterenol resulted in a decreased beta adrenergic responsiveness and loss of beta adrenoceptors. However, exposure of human adipocytes to epinephrine did not alter the alpha$_2$ adrenergic responsiveness or the number of alpha$_2$ adrenergic receptors (82). Why interaction of inhibitory receptors with the cyclase did not produce desensitization is unclear.

E. Alpha$_2$ catecholamine inhibition of cyclic AMP and lipolysis in hamster and human adipocytes. The first report on alpha adrenergic inhibition of human adipocyte lipolysis was that of Burns and Langley (83) in 1970. Subsequently it was demonstrated that cyclic AMP levels were reduced by alpha adrenergic stimulation in adipocytes (50, 51, 53–55, 65, 84) as well as adenylate cyclase activity of fat cell ghosts from hamster or human adipocytes (46, 47, 52, 53, 66). Adipocytes from dogs and rabbits (15) also have alpha$_2$ adrenoceptors but rat adipocytes do not seem to have functional alpha$_2$ adrenoceptors (51, 85). In addition to this difference between species there is also variation in the alpha$_2$ adrenergic sensitivity of adipocytes from different regions of the body (15, 86). Furthermore, the alpha$_2$ adrenergic responsiveness and the number of alpha$_2$ adrenoceptors seem to increase with age in hamster adipocytes (87). The physiological significance of these variations remains to be established. Alpha$_2$ adrenergic activation antagonizes beta adrenergic activation of phosphorylase (49) and stimulation of glucose oxidation (88) in hamster

[2] Moreno, F. J., I. Mills, J. N. Fain, and J. A. García-Sáinz. *J. Biol. Chem.* In press.

[3] García-Sáinz, J. A. Unpublished observations.

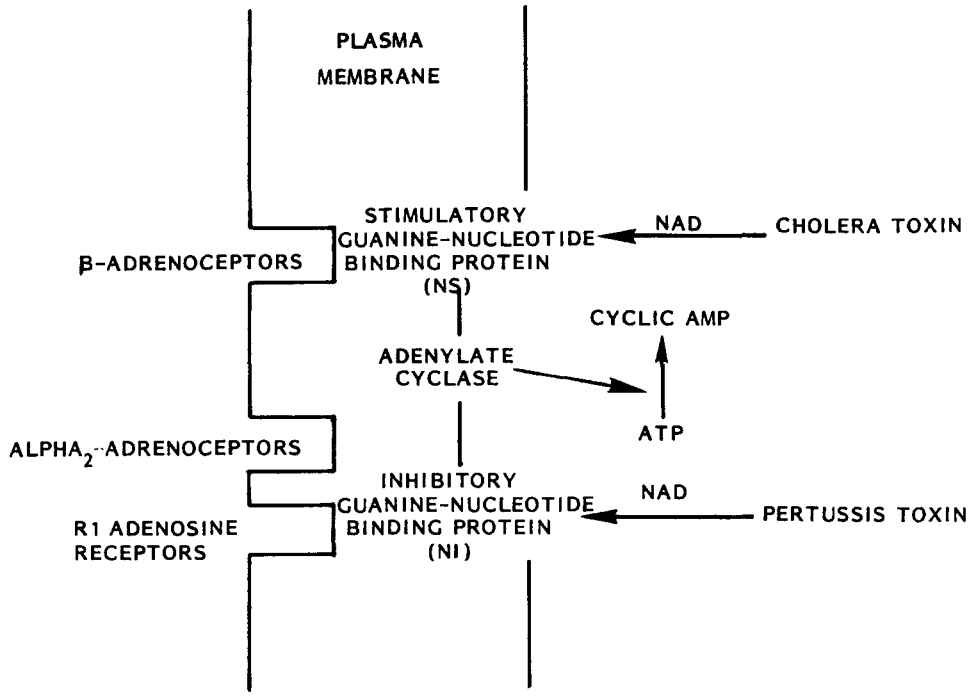

Fig. 5. Regulation of adenylate cyclase by cholera toxin and pertussis toxin.

adipocytes. It is reasonably clear, therefore, that alpha$_2$ and beta adrenoceptors mediate opposing effects on the metabolism of hamster and human adipocytes. The balance between these receptors could be a point of regulation that determines the final effect of the natural catecholamines on adipocytes in vivo.

Kather and Schroder (89) found that adipocytes isolated from the hypertrophic adipose tissue of humans with multiple symmetric lipomatosis had a normal alpha$_2$ and beta-adrenergic responsiveness with respect to lipolysis. Their studies were made possible by the development of a sensitive bioluminescent assay for glycerol, permitting multiple samples to be assayed using the limited number of adipocytes that can be isolated from needle biopsies of humans (90). A normal adrenergic responsiveness agrees with the finding of an unchanged adenylate cyclase sensitivity to beta catecholamines in multiple symmetric lipomatosis (91, 92). One report, based on a single subject, had noted a complete block in the lipolytic action of catecholamines (93). However, this might reflect some of the normal variations encountered in studies on human adipocyte lipolysis. Thus, the bulk of the evidence is for unchanged sensitivity to catecholamines in multiple symmetric lipomatosis.

F. Identification of alpha$_2$ adrenoceptors in adipocytes. Pecquery, Malagrida, and Giudicelli (94) initially reported the identification of alpha adrenoceptors in hamster adipocytes using [^{3}H]dihydroergocryptine which was later confirmed by us and others (51, 53, 95, 96). Dihydroergocryptine bound to a homogeneous class of receptors with a B_{max} in the range 300–1200 fmol/mg protein and K_D in the range 2.5 to 15 nM. Later, it was reported that dihydroergocryptine binding was biphasic due to two types of receptors with different affinities for this ligand (97). In our hands, dihydroergocryptine binding was to a homogeneous class of receptors of indistinguishable affinity (51, 53, 95). Competition displacement studies on [^{3}H]dihydroergocryptine binding by the selective ligands, prazosin and yohimbine, revealed the presence of both alpha$_1$ and alpha$_2$ adrenoceptors in hamster adipocytes (51). Alpha$_1$ adrenoceptors represented 20–30% of the sites whereas alpha$_2$ adrenoceptors represented the great majority of the sites (70–80%). Similar proportions of sites have been obtained by others in hamster adipocytes (97, 98). The large variations in the number of alpha$_2$ adrenoceptors may be due to differences in the preparation of membranes and the age of the animals employed; i.e., the number of alpha adrenoceptors in hamster adipocyte membranes changes from 180 fmol/mg of protein at 45 days of age to 1700 fmol/mg protein at 10 months (87). Guanine nucleotides do not affect either the binding of dihydroergocryptine nor its displacement by alpha adrenergic antagonists (99). However, guanine nucleotides severely decrease the overall binding affinity of alpha$_2$ adrenoceptors for agonists (99), indicating that guanine nucleotides promote the conversion of the high

affinity state of the receptor to a low affinity state. Studies on the binding of [^{3}H]norepinephrine to alpha adrenoceptors are in agreement with those on antagonist binding to hamster adipocytes (98).

Direct determination of alpha$_2$ adrenergic receptors in human adipocytes has been performed using [^{3}H]dihydroergocryptine, which is a nonsubtype selective alpha antagonist (53). Specific alpha$_2$-selective agonists such as [^{3}H]clonidine (100) or [^{3}H]para-aminoclonidine (53) and [^{3}H]yohimbine, a relatively specific alpha$_2$ antagonist, have been used more recently (101). Numerous discrepancies exist in alpha$_2$ adenoceptor binding data that may be linked to differences in age of the humans from which adipose tissue was obtained, degree of obesity, and even to membrane storage conditions or the composition of buffers used in binding studies. Nevertheless, the ratio of [^{3}H]yohimbine binding sites to [^{3}H]clonidine or [^{3}H]para-aminoclonidine binding sites is always larger than 1, suggesting that alpha$_2$ antagonists label a larger number of sites than alpha$_2$ agonists. Studies on [^{3}H]yohimbine binding demonstrated that two affinity states of the receptor are apparently involved in the binding of alpha$_2$ agonists (101). Furthermore, the affinity of the alpha$_2$ receptor of human fat cells for agonists is decreased in the presence of sodium and guanine nucleotides while that for antagonists is unaffected (101, 102).

V. Elevation of cytosol Ca^{2+} and phosphatidylinositol turnover via alpha$_1$ receptors

A. Introduction. In the initial burst of enthusiasm after the discovery of cyclic AMP, all effects of catecholamines were attributed to this second messenger. However, in studies in our laboratory on gluconeogenesis in rat liver hepatocytes, it was found that alpha adrenergic antagonists markedly reduced the increase in glycogen phosphorylase due to epinephrine without affecting catecholamine stimulation of cyclic AMP accumulation (103). Previously Sherline, Lynch, and Glinsmann (104) had noted similar findings with perfused rat liver but thought that the alpha catecholamine effects were due to vasoconstrictors that elevated AMP. Subsequent studies have confirmed that alpha catecholamine effects on both glycogen phosphorylase and gluconeogenesis involve some second messenger other than cyclic AMP (105–110).

Studies from many different laboratories have suggested that Ca^{2+} rather than cyclic AMP or cyclic GMP is the second messenger for alpha catecholamine stimulation of glycogenolysis and gluconeogenesis in rat hepatocytes. Several reviews of this subject have appeared during the past few years (41, 111–114). However, the mechanisms by which alpha catecholamines elevate intracellular Ca^{2+} remain to be elucidated. In rat hepatocytes, under physiological conditions, all alpha effects of catecholamines are mediated through alpha$_1$ receptors (115–118).

There are alpha$_1$ catecholamine effects on phosphatidylinositol turnover in hepatocytes (115) and adipocytes (119, 120). In fact, in almost every cell in which a transmembrane signal is believed to elevate Ca^{2+}, there is also an increased turnover of phosphatidylinositol (12, 121). The question of whether there is a causal relationship between these two events is being actively investigated at this time. One group believes that phosphatidylinositol turnover is secondary to elevations in cytosol Ca^{2+} (122); another group believes that hormones accelerate the breakdown of polyphosphoinositides which causes the release of bound Ca^{2+} (123); while we think that hormones directly activate phosphatidylinositol breakdown, which is linked in some unknown way to the elevation in cytosol Ca^{2+}, but is not secondary to elevations in Ca^{2+} (12, 124).

B. Alpha$_1$ adrenoceptors in adipocytes. Alpha$_1$ adrenoceptors have been detected using ^{3}H-labeled WB4101 (B_{max}, 303 fmol/mg of protein; K_D, 0.86 nM) in human adipocyte membranes (53). They have also been detected indirectly using prazosin displacement of other ligands ([^{3}H]dihydroergocryptine and [^{3}H]-norepinephrine) in hamster adipocyte membranes (51, 97, 99). The alpha$_1$ adrenoceptors represent 20–30% of the total alpha adrenergic receptors.

Activation of alpha$_1$ adrenoceptors increases the labeling of phosphatidylinositol and its precursor phosphatidic acid in hamster (51), human (53), or rat adipocytes (119, 120) and modulates glycogen metabolism (120, 125, 126). However, attempts to directly demonstrate alpha adrenoceptors in rat fat cell membranes have been unsuccessful. There are sites labeled with prazosin or dihydroergocryptine in rat adipocyte membranes (127). The binding is rapid, reversible, saturable, and of high affinity but the sites do not have all the pharmacological properties expected in alpha adrenoceptors (127). The authors concluded that true alpha adrenoceptors do not exist in rat fat cells and that the binding sites they detected represented phylogenetic remnants of true alpha-adrenoceptors (127). The pharmacological characteristics of the phosphatidylinositol effect in rat fat cells indicate that the receptor involved has the expected characteristics for alpha$_1$ adrenoceptors (119, 120). However, the direct determination of alpha$_1$ adrenoceptors in rat fat cells remains a challenging goal.

C. Alpha$_1$ catecholamine effects on adipoctye glycogen metabolism. There are alpha$_1$ catecholamine effects on adipocytes from human, rat, hamster, sheep, and other species. The physiological meaning of these effects is unclear since the effects have little to do with regulation

of lipolysis. Rather it is the enzymes of glycogen metabolism that are affected by alpha catecholamines as depicted in **Fig. 6.** Lawrence and Larner (125, 126) found that alpha catecholamines activate glycogen phosphorylase and inactivate glycogen synthase in rat adipocytes.

The most likely mechanism for regulation of these enzymes by alpha catecholamines is through elevation of cytosol Ca^{2+}. Khoo (128) found that the phosphorylase kinase of adipose tissue is a Ca^{2+}-dependent enzyme which suggests this is a site for Ca^{2+} action. Lawrence and Larner (126) found that the alpha-adrenergic inactivation of glycogen synthase was not seen in the absence of extracellular Ca^{2+} and was mimicked by the divalent cation ionophore A-23187. The inactivation of rat adipocyte glycogen synthase by catecholamines has been shown to be an $alpha_1$ effect (120). However, in hamster and human adipocytes a decrease in cyclic AMP accumulation is seen in response to $alpha_2$ adrenergic stimulation which would activate glycogen synthase. Insulin has effects on glycogen synthase which do not appear to be adequately explained by changes in cyclic AMP or Ca^{2+} (126).

The role of Ca^{2+} in regulation of adipocyte metabolism is rather unclear except for the enzymes of glycogen metabolism. One of the key regulators of intracellular Ca^{2+} dynamics is the plasma membrane ATPase which presumably pumps Ca^{2+} out of cells. A direct inhibitory effect of insulin on this enzyme has been reported in adipocyte plasma membranes (129). This would presumably elevate intracellular Ca^{2+} just as $alpha_1$ adrenoceptor activation is postulated to do. Since insulin and $alpha_1$ catecholamines have opposite effects on glycogen synthase there must be other factors involved in insulin action.

The known effects of $alpha_1$ adrenoceptor activation on rat adipocytes are shown in **Table 4.** Only the inactivation of glycogen synthase and ^{32}P uptake into phosphatidylinositol have been shown to be $alpha_1$ catecholamine effects (120). Since there is general agreement that no $alpha_2$ adrenoceptors or responses exist in rat adipocytes, it is probable that K^+ efflux and activation of glycogen phosphorylase are $alpha_1$ effects.

D. Insulin vs. catecholamine effects on phosphatidylinositol turnover. Catecholamines increase ^{32}P uptake into phosphatidylinositol via $alpha_1$ adrenoceptor activation in

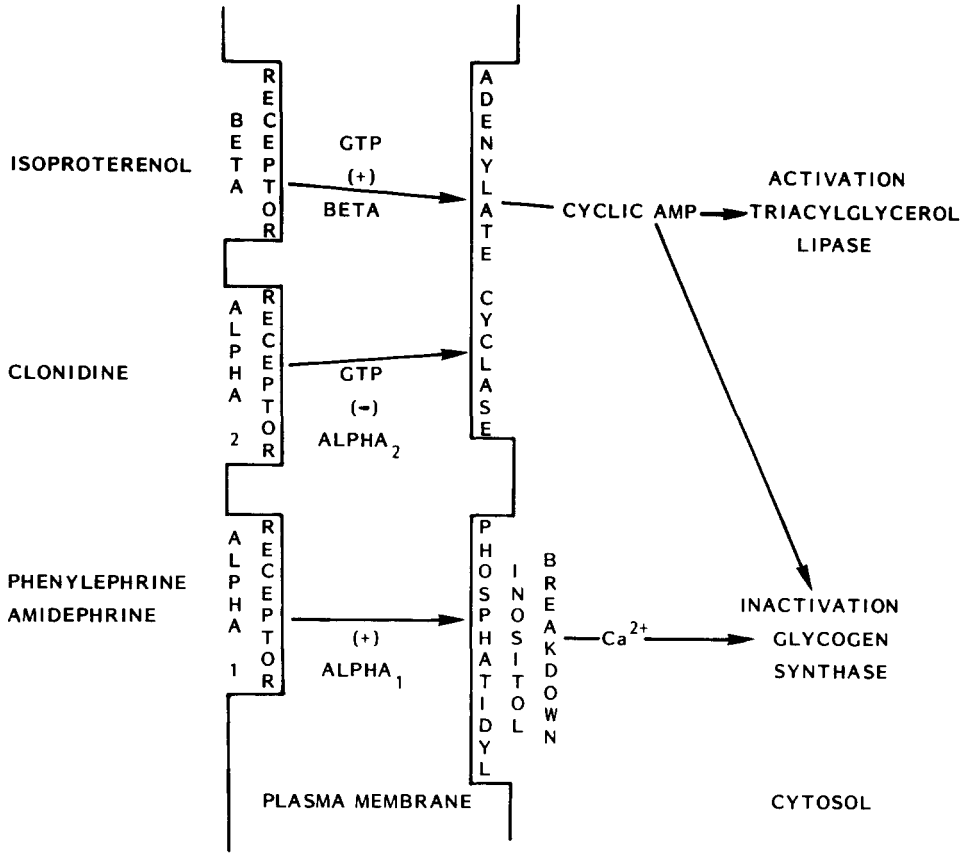

Fig. 6. Effects of catecholamines on adipocytes. Synthetic amines which are relatively selective for each type of receptor are shown on the left and the two major responses in adipocytes on the right. Cyclic AMP can also inactivate glycogen synthase, but Ca^{2+} has little effect on the triacylglycerol lipase.

TABLE 4. Effects on rat adipocytes attributed to activation of alpha$_1$ adrenoceptors

Effect	Mechanism	Reference
Stimulation of $^{42}K^+$ efflux	Elevated cytosol Ca^{2+}	Perry and Hales (130)
Activation of glycogen phosphorylase	Elevated cytosol Ca^{2+}	Lawrence and Larner (125)
Inactivation of glycogen synthase	Elevated cytosol Ca^{2+}	Lawrence and Larner (126), García-Sáinz and Fain (120)
Increase in ^{32}P uptake into phosphatidylinositol and phosphatidic acid	May be secondary to release of Ca^{2+} from the endoplasmic reticulum to cytosol	Stein and Hales (131), García-Sáinz and Fain (119)

human (53), hamster (51), and lamb perirenal adipocytes.[4] The relationship between this event and the elevation of cytosol Ca^{2+} is not known. However, in cells in which alpha$_1$ catecholamines, muscarinic cholinergic, or other agents elevate Ca^{2+}, an increased uptake of ^{32}P into phosphatidylinositol can be readily observed (12, 121).

Insulin stimulated the labeling with [^{32}P]P_i of all phospholipids in rat adipocytes (119). However, if adipocytes were incubated in Ca^{2+}-free medium containing EGTA, the effect of insulin was specific for labeling of phosphatidylinositol and phosphatidic acid; interestingly, ionophore A23187 mimicked this effect (119). If 2.5 mM $CaCl_2$ was added to the buffer containing EGTA, there was still a stimulation of phosphatidylinositol labeling by alpha$_1$ catecholamines but the effects of insulin and A23187 were reversed and these agents now slightly inhibited phosphatidylinositol labeling (119). One explanation for these results is that in the absence of extracellular Ca^{2+} all three agents cause net movement of Ca^{2+} out of the endoplasmic reticulum which stimulated phosphatidylinositol synthesis. In contrast, if Ca^{2+} was present in the medium, net movement due to insulin and A23187 could be in the opposite direction, resulting in increases in Ca^{2+} content of the endoplasmic reticulum. Fain (12) has suggested that it is the level of Ca^{2+} in the endoplasmic reticulum that regulates phosphatidylinositol synthesis.

The effects of insulin on phospholipid turnover are most complex and today there is more confusion than clarity. We do not know why insulin can mimic the effect of alpha catecholamines on phosphatidylinositol turnover in the absence of extracellular Ca^{2+} but not in the presence of Ca^{2+} and EGTA. Kiechle et al. (132) have recently suggested that phosphatidylserine might be released into the cytosol after interaction of insulin with plasma membrane receptors. This was based on the finding that this phospholipid mimicked effects of insulin on activation of adipocyte cyclic AMP phosphodiesterase and pyruvate dehydrogenase.

[4] Mohell, N., and J. N. Fain. Unpublished studies.

Even more interesting was the report that after less than 30 min incubation with insulin, the net level of total phosphatidylinositol, polyphosphoinositides, and phosphatidic acid in adipocytes was increased by 50% (133). These results could be explained by an increased synthesis or a decreased breakdown; however, it is hard to imagine how insulin exerts effects large enough to account for a 50% increase in 30 min. These findings will serve as a stimulus for an extensive investigation of insulin effects on phospholipid metabolism in adipocytes.

Michell et al. (123) and Fain (12) have pointed out that measuring effects of alpha$_1$ catecholamines and other agents on ^{32}P uptake into phospholipids is relatively easy but is probably a secondary event. Ideally inferences about the relationship between phosphatidylinositol breakdown and the release and/or entry of Ca^{2+} into the cytosol should be based on direct measurement of breakdown. There are several problems that complicate studies on the breakdown of plasma membrane phosphatidylinositol in response to hormones. If the hypothesis is correct that breakdown of plasma membrane phosphatidylinositol is linked to elevation of intracellular Ca^{2+}, then it would appear likely that only a small fraction of the total plasma membrane phosphatidylinositol is broken down in response to hormone. Furthermore, the plasma membrane pool is only a small fraction of total cellular phosphatidylinositol. In fly salivary glands previously labeled by incubation with arachidonic acid (134), inositol (135, 136), or ^{32}P (134–136), most of the newly synthesized phosphatidylinositol equilibrated with a small pool which was probably less than 5% of total phosphatidylinositol. After addition of the hormonal stimulus, breakdown of over 80% of the prelabeled phosphatidylinositol was seen without any demonstrable change in total phosphatidylinositol (136). In other systems it has been difficult to preferentially label and separate the plasma membrane pool of phosphatidylinositol.

In hepatocytes isolated from rats injected with tritiated inositol 18 hr previously, there was a breakdown of about 5% of the labeled phosphatidylinositol during

the first 5 min after addition of vasopressin (123, 137). Lin and Fain (137) found that the breakdown of prelabeled phosphatidylinositol in the plasma membrane-rich fractions was about 16 to 19% after exposure to vasopressin for 5 min but there was no breakdown of phosphatidylinositol in the endoplasmic reticulum. Recently it was found that the direct addition of vasopressin to rat liver plasma membranes resulted in an increased disappearance of total phosphatidylinositol (124, 138). Furthermore, the effect was independent of Ca^{2+} since the studies were done in the complete absence of Ca^{2+} as the membranes were incubated in the presence of 0.5 mM EGTA. Harrington, Davis, and Eichberg (139) obtained similar results after the addition of norepinephrine to rat liver plasma membranes. These results suggest that either the hormone is activating a Ca^{2+}-independent phospholipase C or is increasing accessibility of phosphatidylinositol to an endogenous phospholipase C present in the Neville preparations of liver plasma membranes.

In adipocytes it has as yet been impossible to get enough label into phosphatidylinositol under the conditions used for labeling hepatocyte phosphatidylinositol. It should be possible to obtain direct stimulation of phosphatidylinositol breakdown of adipocyte plasma membrane after addition of $alpha_1$ catecholamine agonists. However, the available methods for determination of total phosphatidylinositol breakdown are not sensitive enough to permit these experiments to be readily done.

The five major effects of $alpha_1$ catecholamines are summarized in **Fig. 7.** Entry of extracellular Ca^{2+} is not required for $alpha_1$ catecholamine activation of glycogen phosphorylase in hepatocytes (107, 115, 140). Whether this is also true in adipocytes is unclear. However, the other four effects are generally seen after hormone addition and the figure suggests the most probable sequence of events. Question marks are placed by each arrow to indicate that the scheme outlined is a hypothesis.

VI. Regulation of adrenergic sensitivity by thyroid hormone

Thyroid hormones modulate adipocyte responsiveness to catecholamines. Adipocytes from hyperthyroid animals display an increased sensitivity to all lipolytic hormones including catecholamines and the opposite is true for cells from hypothyroid animals (17, 18, 141).

The effect of thyroid status on the alpha-adrenergic responsiveness of adipocytes is unclear. Rosenqvist et al. (142) suggested that hypothyroidism resulted in a marked shift in the balance between alpha and beta adrenergic receptors in human adipose tissue. Giudicelli, Lacasa, and Agli (143) reported that hyperthyroidism decreased by 35–45% the number of alpha adrenoceptors in hamster adipocytes. In contrast, we observed no reduction in the number of alpha adrenoceptors in fat cells of hyperthyroid hamsters or of the $alpha_2$ adrenergic responsiveness (95).

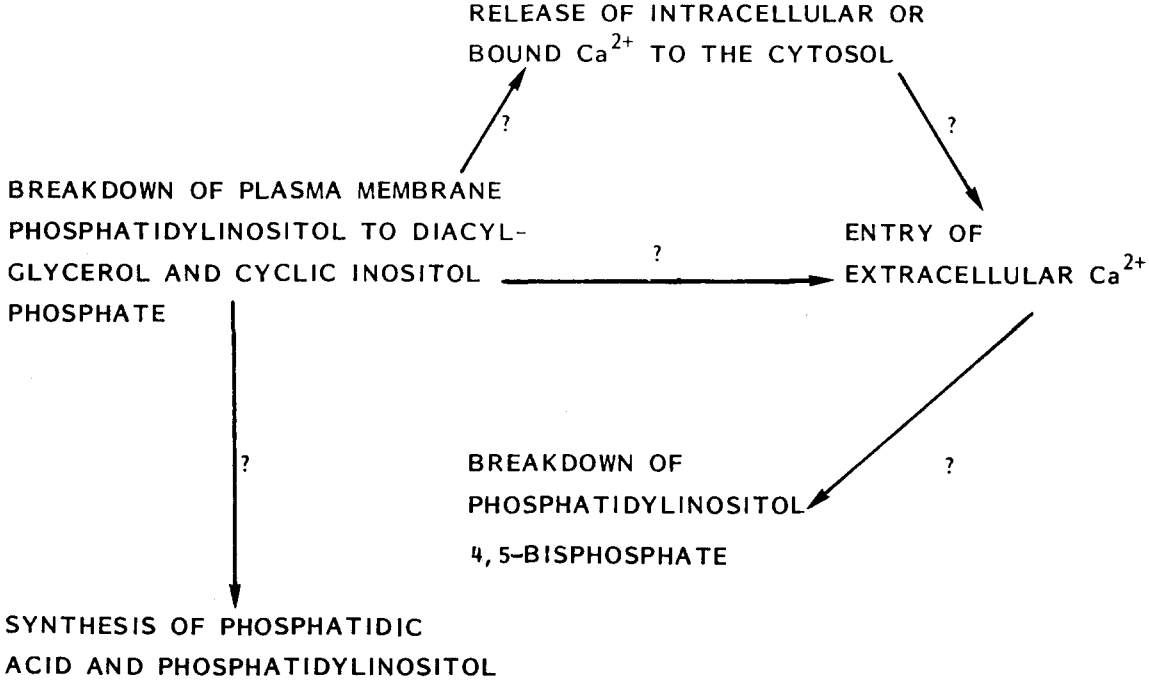

Fig. 7. Events involved in $alpha_1$ catecholamine action on target cells.

The defective accumulation of cyclic AMP observed in adipocytes from hypothyroid rats is probably due to an impaired beta adrenergic response rather than to changes in alpha responses. Rats do not have functional $alpha_2$ adrenoceptors in their adipose tissue and there are no known effects of $alpha_1$ adrenoceptors on lipolysis (17, 18, 141). $Alpha_1$ adrenoceptors do regulate adipocyte glycogen synthase. The inhibition by beta catecholamines as well as the stimulation by insulin of glycogen synthase was markedly reduced in adipocytes from hypothyroid rats (120). In contrast, the $alpha_1$ adrenoceptor inactivation of glycogen synthase was little affected by thyroid status.

The mechanism by which thyroid hormones regulate lipolytic sensitivity is not yet established. Since sensitivity to all hormones is affected, it is unlikely that thyroid hormones specifically regulate the number of beta adrenoceptors (17, 141). In agreement with this, Malbon et al. (144) found little effect of thyroid status on the number or affinity of binding sites for [^{3}H]dihydroalprenolol, a beta-adrenergic antagonist.

The ability of catecholamines to elevate cyclic AMP accumulation is markedly reduced in adipose tissue from hypothyroid animals (141). This is probably due to an impaired activation of adenylate cyclase by hormone-receptor complexes (141, 144). There was a reduction in hormonal activation of adenylate cyclase in isolated membranes from hypothyroid rats under some, but not all, conditions (141). This may reflect a differential loss of the factors involved in regulating the interaction of all hormone receptor complexes with adenylate cyclase. A guanine-nucleotide binding protein is involved in this coupling and may be the site of thyroid hormone action. One of the most impressive effects of thyroid status on isolated adipocyte membranes is the change in catecholamine agonist displacement of antagonist binding which was greater in adipocyte membranes from hyperthyroid rats as compared to euthyroid controls. In membranes from hypothyroid rats, agonist displacement was reduced (145). Interestingly, guanine nucleotides did not inhibit catecholamine displacement of antagonist (dihydralprenolol) binding in membranes from hypo- or hyperthyroid rats as was seen in membranes from euthyroid controls (145). Much remains to be learned about how thyroid hormones regulate adenylate cyclase.

The increase in cyclic AMP phosphodiesterase activity seen in adipocytes from hypothyroid rats is probably due to a decrease in growth hormone secretion. Peake, Birge, and Daughaday (146) found that growth hormone levels were hardly detectable in serum from hypothyroid rats. This might explain the increased hexose transport activity (147, 148) and cyclic AMP phosphodiesterase activity (149, 150) seen in hypothyroid rats. Similar increases are seen in hypophysectomized rats and are normalized by growth hormone treatment (151, 152). Schoenle, Zapf, and Froesch (151) found that thyroid hormone administration to hypophysectomized rats did not affect the elevated cyclic AMP phosphodiesterase activity observed in adipocyte homogenates. These data suggest that thyroid hormone effects on cyclic AMP phosphodiesterase are mediated through growth hormone.

It is possible that thyroid hormones might regulate the ratio of stimulatory to inhibitory guanine-nucleotide binding proteins shown in Fig. 5. In isolated adipocyte membranes from hypothyroid rats there appeared to be more N_s protein which could be ADP-ribosylated by cholera toxin (153). Pertussis toxin markedly increases lipolysis and cyclic AMP due to forskolin or catecholamines in adipocytes from hypothyroid rats.[5]

VII. Effects of obesity, age, and nutritional status on adrenergic responsiveness

The cyclic AMP response to epinephrine and norepinephrine can depend on the relative ratio of $alpha_2$ to beta effects. In adipocytes taken from obese individuals fasted for 3 days, there was a reduction in epinephrine-induced lipolysis and cyclic AMP accumulation (154). This reduction was attributed to a decrease in beta adrenoceptor activation of lipolysis rather than to an increase in $alpha_2$ processes (154).

The influence of obesity on the ratio of alpha to beta adrenoceptor activity has not been carefully examined in humans. However, no inhibitory $alpha_2$ responses can be seen on adipocytes from normal dogs, while a marked anti-lipolytic effect of $alpha_2$ adrenoceptor activation can be seen in cells from obese dogs (155). Similar results have been seen in old obese rabbits (15).

In subcutaneous adipose tissue obtained from obese humans after a 7-day fast, the addition of norepinephrine actually reduced basal cyclic AMP by 23% and lipolysis by 24% (156). In adipocytes obtained from the same individuals just prior to onset of the fast, the addition of the same amount of norepinephrine elevated cyclic AMP by 68% and lipolysis by 66%. The inhibition of basal cyclic AMP by norephinephrine was greatest in the tissues with the smallest size adipocytes. These data were based on incubation of adipose tissue in the absence of albumin and presence of 10 mM theophylline for the cyclic AMP studies. Obese humans have a substantial $alpha_2$ inhibitory response to norepinephrine or epinephrine and starvation apparently reduces beta ad-

[5] Mills, I., J. N. Fain, and J. A. García-Sáinz. Unpublished experiments.

renoceptor sensitivity much more than alpha adrenoceptor sensitivity (154, 156).

Regional differences exist in the ratio of alpha to beta adrenoceptors in humans (86, 157, 158). There is a smaller $alpha_2$ component in regulation of lipolysis and cyclic AMP accumulation in omental as compared to epigastric subcutaneous adipose tissue of fed humans (157). There was also a more marked $alpha_2$ inhibitory component in femoral than in hypogastric subcutaneous adipose tissue after a 7-day fast in morbidly obese humans (157). These regional differences suggest that it might be possible through the use of $alpha_2$ agonists and antagonists to affect preferential loss of certain lipid depots during starvation.

An increased level of $alpha_2$ adrenoceptor activity could be a causative feature in the development of obesity but this is a rather speculative hypothesis. Curtis-Prior (159) suggested a somewhat similar hypothesis in 1975 in which overproduction of prostaglandins inhibited lipolysis in individuals prone to obesity. Prostaglandins, like adenosine and $alpha_2$ adrenergic agonists, are all thought to work through an inhibitory guanine-nucleotide binding protein (Fig. 5) to inhibit adipocyte adenylate cyclase (46, 47, 63). There is little evidence that prostaglandins are physiological regulators of adipocyte lipolysis during short-term studies (17, 18, 79). It has also been difficult to prove that adenosine is a physiological regulator of lipolysis (17, 18, 78–80). However, all three agents are potent inhibitors of adenylate cyclase and anything that increases their levels in adipose tissue should theoretically inhibit lipolysis. Recently we found that in adipocytes from rats or hamsters treated with pertussis toxin, basal lipolysis was elevated and the sensitivity of the cells to forskolin and catecholamines was increased 10- to 100-fold.[2] Pertussis toxin blocked the inhibition of lipolysis by adenosine, prostaglandins, and $alpha_2$ catecholamines.[3]

The effects of aging on adipocyte metabolism are often difficult to dissociate from the effects of obesity. Most studies have been done with male rats from strains that have a progressive increase in fat cell size and number with age. In one study, the number of binding sites, but not the affinity, for the beta catecholamine antagonist dihydroalprenolol changed with age (160). In 1-month-old rats there were approximately 11,000 binding sites per cell which increased to 40,000/cell at 8 months and then slowly declined to 7,000 sites per cell at 24 months (160). The log-dose response curves for activation of adenylate cyclase by norepinephrine or isoproterenol reflected the number of receptors per cell. It was less clear what effect aging has on lipolysis, as the response to only a single concentration (0.1 μM) of norepinephrine was tested and the values were expressed per gram of cell lipid rather than per cell. If the lipolytic response to 0.1 μM norepinephrine was expressed per cell, there was a dramatic decline in the adipocytes from the 8-month-old rats as compared to those from 1-month-old rats and no lipolysis in adipocytes from 30-month-old rats (160).

Some effects of aging have been separated from those of obesity (161, 162). In male Fischer rats maintained on a restricted food intake, longevity is increased by 49% and adipocyte hypertrophy is not seen. At 6 months of age, there was a similar lipolytic response to catecholamines in both groups (162). However, by 12 months of age there was a selective loss in catecholamine responsiveness in adipocytes from the obese rats fed ad libitum (162). In adipocytes from rats whose food intake was restricted, there was a much smaller loss of responsiveness to catecholamines with age (162).

Dax, Partilla, and Gregerman (163) found no loss in adenylate cyclase responsiveness to epinephrine with aging. They used male Wistar rats and, even in adipocytes isolated from 24-month-old rats (the median life span for this strain fed on laboratory chow), there was no change in beta-adrenergic antagonist binding sites, maximum cyclic AMP accumulation, or adenylate cyclase activity as compared to adipocytes from 6- to 12-month-old rats. The adipocytes from the 24-month-old rats were rather fragile and if centrifuged during isolation there was considerable breakage. The only effect of aging was to markedly decrease maximum lipolysis in response to epinephrine (163). It has been suggested that some of the changes with aging in lipolytic sensitivity of adipocytes from mice were due to changes in fat cell size (164). However, in the studies of Dax et al. (163), this did not account for the changes in lipolytic sensitivity to epinephrine noted at 24 months.

The effects of aging appear to be rather variable. However, a large part of this may be due to differences in species, strains, and diet as well as failure to dissociate changes due to obesity in rodents from those of aging. Furthermore, the marked fragility of adipocytes from senescent rodents may influence results as the products released during cell lysis can have deleterious effects on the surviving adipocytes. There does appear to be a decrease in lipolytic sensitivity of adipocytes from senescent rats to epinephrine (160, 163) which cannot be explained by decreases in the ability of catecholamine-receptor complexes to activate adenylate cyclase. In effect, there is a dissociation between cyclic AMP formation and lipolysis in adipocytes from senescent rats. The defect is either in the ability of cyclic AMP to activate lipolysis or in unknown factors involved in lipolytic regulation.

Starvation of rats has effects similar to those of aging and obesity on catecholamine-induced lipolysis. Male rats (140–160 g) were starved for 72 hr, which resulted

in loss of 40–65% of their adipose tissue weight (165). Crude membrane preparations derived from adipocytes had more beta adrenoceptors and were more sensitive to activation of adenylate cyclase by isoproterenol per mg of protein. However, the lipolytic response of adipocytes from starved rats was markedly reduced on a per cell basis to isoproterenol, ACTH, and dibutyryl cyclic AMP (165). Thus, starvation of the rat, like aging, uncoupled adenylate cyclase activation from lipolysis.

Hopefully, future studies will examine the effects of nutritional status and aging on alpha receptors and responsiveness. One problem in such studies is that it is not yet clear how to measure $alpha_1$ adrenoceptors in rat adipose tissue, as there are problems with dihydroergocryptine and prazosin binding (127). While it is possible to use dihydroergocryptine for measurement of alpha adrenoceptors in hamster adipocytes (51, 96, 97), it is rather difficult to obtain yohimbine binding to white[6] or brown[7] adipocytes.

Clonidine is quite effective in inhibiting lipolysis and cyclic AMP accumulation in intact hamster (95, 166) and human (53, 54) adipocytes. While clonidine is always more potent than epinephrine in displacing labeled yohimbine binding from human adipocyte ghosts (102), it has been reported to be a poor inhibitor of adenylate cyclase activity in adipocyte membranes from hamsters. Furthermore, in the presence of guanine nucleotides and high NaCl concentration, the ability of epinephrine to displace yohimbine binding was reduced 100-fold (102). In contrast, clonidine displacement of yohimbine binding was reduced only 6-fold by guanine nucleotides in the presence of NaCl. These discrepancies point out the variations encountered in studies on the binding of labeled agonist and antagonists from one species to the next and possibly even in different types of adipose tissue within a given animal. A further problem is that the only thing that can be readily measured is high affinity binding sites for antagonists. The binding of labeled hormones to membranes is reduced by guanine nucleotides that are also required for coupling of the hormone receptor complexes to adenylate cyclase as first pointed out by Rodbell and associates (167, 168). This suggests that the highest affinity site for binding may be one in which receptors are not linked to any function, while the interaction of hormone-receptor complex with guanine-nucleotide binding protein in the presence of GTP results in activation of adenylate cyclase as well as a reduction in binding affinity for the hormone.

VIII. Effects of adrenoceptor activation in brown adipocytes

Brown adipocytes differ from white adipocytes in having multiple lipid droplets and many mitochondria with tightly packed cristae (169). The major function of brown fat is thermogenesis due to oxidation by the abundant mitochondria of fatty acids released during lipolysis (169). In brown fat mitochondria, oxidative phosphorylation is readily uncoupled by free fatty acids. The sequence for thermogenesis is beta adrenoceptor activation of adenylate cyclase, increased phosphorylation of triacylglycerol lipase, and finally, increased respiration due to oxidation of free fatty acids released during lipolysis.

In brown adipocytes from hamsters there are $beta_1$ adrenoceptors that bind dihydroalprenolol (170). There are also alpha adrenoceptors that bind dihydroergocryptine (171) and prazosin (172). The latter ligand labels only $alpha_1$ adrenoceptors. There are $alpha_1$ effects on uptake of ^{32}P into phosphatidylinositol (172–174) and on respiration in adipocytes isolated from brown fat of hamsters (172). The evidence for $alpha_2$ catecholamine responses in hamster brown adipocytes is less clear. One group could detect no effects (175), while others saw an inhibition of lipolysis and cyclic AMP accumulation (176).

Thyroid hormones affect catecholamine responsiveness of brown adipocytes. In adipocytes from hypothyroid mice the stimulation of respiration by catecholamines (177) and the state of pyridine nucleotide reduction after catecholamine addition to adipocytes from hypothyroid rats (178) were markedly reduced. Hypothyroid rats are unable to maintain sufficient nonshivering thermogenesis to survive cold exposure (179, 180).

The best explanation for the data is that thyroid hormones have opposite effects on thermogenic capacity in brown fat as opposed to catecholamine sensitivity. Indeed, Seydoux, Giacobino, and Girardier (178) found that the metabolic response to octanoate was enhanced by hypothyroidism. This agrees with the finding that GDP binding was enhanced in cells from hypothyroid rats where the reduction in overall basal metabolic rate is compensated by an increase in brown fat thermogenesis (181). In hypothyroid rats there is an increase in thermogenin and of heat production by brown fat to compensate for the general decrease in basal metabolic rate seen in other tissues (181). This raises the question of what regulates thermogenesis at ambient temperatures in brown fat. If it is catecholamines, the rate of their release must be greatly accelerated in hypothyroid animals. It is unclear how thyroid hormones regulate the amount of mitochondrial thermogenin that binds GDP and apparently compensates for the reduced sensitivity of these animals to catecholamines. The data suggest that thyroid hormone regulation of catecholamine sensitivity is relatively unimportant in regulation of mitochondrial thermogenesis except during cold adaptation (181, 182).

[6] Lafontan, M. Personal communication.
[7] Mohell, N. Personal communication.

Currently there is a lot of interest in diet-induced thermogenesis in brown fat. The administration of a diet high in carbohydrate and low in protein induces hypertrophy of brown fat in rodents and an increase in catecholamine-induced thermogenesis (183). A role for thyroid hormone has been suggested since plasma levels of triiodothyronine were 27% higher in rats fed a hypercaloric diet (184). This may play a role in increased catecholamine sensitivity but some other factor is probably responsible for the increase in mitochondrial thermogenesis. Brown fat mitochondria from rats fed a high-carbohydrate diet bound three times more purine nucleotides than mitochondria from control animals (184). The dietary-induced changes were originally seen after feeding rats a "junk-food diet" consisting of highly palatable food items to humans such as cookies, sandwiches, and sugar-coated cereals. It is now clear that this results in a diet for rodents that is deficient in protein and high in carbohydrate, thus requiring extra caloric intake in order to obtain sufficient protein for growth (184). It is sufficient to supplement the ordinary diet of rodents with sugar in the drinking water to reproduce the changes with the "junk-food diet" (185).

Conclusion

Only recently has it been realized that there are alpha$_1$ and alpha$_2$ effects of catecholamines on adipose tissue. Alpha$_2$ effects are linked to regulation of adenylate cyclase as are the beta effects. In contrast, alpha$_1$ responses are linked to Ca^{2+} but have little effect on lipolysis. However, much remains to be learned about the physiological role of alpha adrenoceptors and especially in human adipocytes.

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REFERENCES

1. Ahlquist, R. P. 1948. A study of the adrenotropic receptors. *Am. J. Physiol.* **153:** 586–600.
2. Robison, G. A., R. W. Butcher, and E. W. Sutherland. 1971. Cyclic AMP. Academic Press, New York.
3. Lands, A. M., A. M. Arnold, J. P. McAuliff, F. P. Luduena, and T. G. Brown. 1967. Differentiation of receptor systems activated by sympathomimetic amines. *Nature.* **214:** 597–598.
4. Minneman, K. P., R. N. Pittman, and P. B. Molinoff. 1981. Beta-adrenergic receptor subtypes: properties, distribution, and regulation. *Annu. Rev. Neurosci.* **4:** 419–461.
5. Ariens, E. J., A. J. Beld, J. F. R. Miranda, and A. M. Simonis. 1979. The pharmacon-receptor-effector concept. *In* Receptors. Vol. 1, General Principles and Procedures. R. D. O'Brien, editor. Plenum Press, New York. 33–91.
6. Berthelsen, S., and W. A. Pettinger. 1977. A functional basis for classification of alpha-adrenergic receptors. *Life Sci.* **21:** 595–606.
7. Wikberg, J. E. S. 1979. The pharmacological classification of adrenergic alpha-1 and alpha-2 receptors and their mechanisms of action. *Acta Physiol. Scand* **Suppl. (468).** 1–99.
8. Fain, J. N., and J. A. García-Sáinz. 1980. Role of phosphatidylinositol turnover in alpha-1 and of adenylate cyclase inhibition in alpha-2 effects of catecholamines. *Life Sci.* **26:** 1183–1194.
9. Bylund, D. B., and D. C. U'Prichard. 1983. Characterization of alpha-1 and alpha-2 adrenergic receptors. *Int. Rev. Neurobiol.* In press.
10. Rasmussen, H. 1981. Calcium and cAMP as Synarchic Messengers. John Wiley & Sons, New York.
11. McGrath, J. C. 1982. Evidence for more than one type of postjunctional alpha-adrenoceptor. *Biochem. Pharmacol.* **31:** 467–484.
12. Fain, J. N. 1982. Involvement of phosphatidylinositol breakdown in elevation of cytosol Ca^{2+} by hormones and relationship to prostaglandin formation. *In* Hormone Receptors. L. D. Kohn, editor. John Wiley & Sons, New York. 237–276.
13. Salzman, E. W., K. C. Kensler, and L. Levine. 1972. Cyclic 3',5'-adenosine monophosphate in human blood platelet. IV. Regulatory role of cyclic AMP in platelet function. *Ann. NY Acad. Sci.* **201:** 61–71.
14. Wollheim, C. B., and G. W. G. Sharp. 1981. Regulation of insulin release by calcium. *Physiol. Rev.* **61:** 914–973.
15. Lafontan, M., and M. Berlan. 1981. Alpha-adrenergic receptors and regulation of lipolysis in adipose tissue. *Trends Pharmacol. Sci.* **2:** 126–129.
16. García-Sáinz, J. A., and J. N. Fain. 1982. Regulation of adipose tissue metabolism by catecholamines: roles of alpha-1, alpha-2, and beta-adrenoceptors. *Trends Pharmacol. Sci.* **3:** 201–203.
17. Fain, J. N. 1980. Hormonal regulation of lipid mobilization from adipose tissue. *In* Biochemical Actions of Hormones, Vol. VII. G. Litwack, editor. Academic Press, New York. 119–204.
18. Fain, J. N. 1982. Regulation of lipid metabolism by cyclic nucleotides. *In* Handbook of Experimental Pharmacology. Vol. 58/II. J. A. Nathanson and J. W. Kebabian, editors. Springer Verlag, Berlin and New York. 89–150.
19. Kather, H. 1981. Hormonal regulation of adipose tissue lipolysis in man: implications for the pathogenesis of obesity. *Triangle.* **20:** 131–143.
20. Fain, J. N. 1973. Biochemical aspects of drug and hormone action on adipose tissue. *Pharmacol. Rev.* **25:** 67–118.
21. Belfrage, P., G. Fredrikson, P. Stralfors, and H. Tornqvist. 1983. Adipose tissue lipases. *In* Lipolytic Enzymes. B. Bergstrom and H. Brockman, editors. Elsevier, Amsterdam.
22. Harms, H. H., J. Zaagsma, and B. Van Der Wal. 1974. Beta-adrenoceptor studies. III. On the beta-adrenoceptors in rat adipose tissue. *Eur. J. Pharmacol.* **25:** 87–91.
23. Harms, H. H., J. Zaagsma, and J. de Vente. 1977. Differentiation of beta-adrenoceptors in right atrium, diaphragm, and adipose tissue of the rat, using stereoisomers of propranolol, alprenolol, nifenalol, and practolol. *Life Sci.* **21:** 123–128.
24. De Vente, J., A. Bast, L. Van Bree, and J. Zaagsma. 1980. Beta-adrenoceptor studies. 6. Further investigations on the hybrid nature of the rat adipocyte beta-adrenoceptor. *Eur. J. Pharmacol.* **63:** 73–83.
25. Kather, H., and B. Simon. 1977. Catecholamine-sensitive adenylate cyclase of human fat cell ghosts: a com-

parative study using different beta-adrenergic agents. *Metabolism.* **24:** 1179–1182.
26. Jacobsson, B., G. Vauquilin, C. Wissiau, U. Smith, and A. D. Strosberg. 1981. Distinction between two subpopulations of beta-1-adrenergic receptors in human adipose cells. *Eur. J. Biochem.* **114:** 349–354.
27. Lai, E., O. M. Rosen, and C. S. Rubin. 1982. Dexamethasone regulates the beta-adrenergic receptor subtype expressed by 3T3-L1 preadipocytes and adipocytes. *J. Biol. Chem.* **257:** 6691–6696.
28. Carlsöö, B., A. Danielsson, R. Henriksson, and L-A. Idahl. 1982. Dissociation of beta-adrenoceptor-induced effects on amylase secretion and cyclic adenosine 3′,5′-monophosphate accumulation. *Br. J. Pharmacol.* **75:** 633–638.
29. Henriksson, R. 1982. Beta-1 and beta-2-adrenoceptor agonists have different effects on rat parotid acinar cells. *Am. J. Physiol.* **242:** G481–G485.
30. Butcher, R. W., and C. E. Baird. 1968. Effects of prostaglandins on adenosine 3′,5′-monophosphate in fat and other tissues. *J. Biol. Chem.* **243:** 1713–1717.
31. Litosch, I., T. H. Hudson, I. Mills, S-Y. Li, and J. N. Fain. 1982. Forskolin as an activator of cyclic AMP accumulation and lipolysis in rat adipocytes. *Mol. Pharmacol.* **22:** 109–115.
32. Seamon, K. B., and J. W. Daly. 1981. Forskolin, a unique diterpene activator of cyclic AMP-generating systems. *J. Cyclic Nucleotide Res.* **7:** 201–224.
33. Moriwaki, K., Y. Itoh, S. Iida, and K. Ichihara. 1982. Forskolin potentiates adrenocorticotropin-induced cyclic AMP production and steroidogenesis in isolated rat adrenal cells. *Life Sci.* **30:** 2235–2240.
34. Yamamura, H., M. Rodbell, and J. N. Fain. 1976. Hydroxybenzylpindolol and hydroxybenzylpropranolol: partial beta-adrenergic agonists of adenylate cyclase in the rat adipocytes. *Mol. Pharmacol.* **12:** 693–700.
35. Fain, J. N. 1977. Cyclic nucleotides in adipose tissue. *In* Cyclic Nucleotides: Mechanisms of Action. H. Cramer and J. Schultz, editors. John Wiley & Sons, London, New York. 207–228.
36. Butcher, R. W., and C. E. Baird. 1969. The regulation of cyclic AMP and lipolysis in adipose tissue by hormones and other agents. *In* Drugs Affecting Lipid Metabolism. W. L. Holmes, L. A. Carlson, and R. Paoletti, editors. Plenum Press, New York. 5–23.
37. Corbin, J. D., E. M. Reimann, D. A. Walsh and E. G. Krebs. 1970. Activation of adipose lipase by skeletal muscle cyclic adenosine-3′,5′-monophosphate-stimulated protein kinase. *J. Biol. Chem.* **245:** 4849–4851.
38. Huttunen, J. K., D. Steinberg and S. E. Mayer. 1970. Protein kinase activation and phosphorylation of purified hormone-sensitive lipase. *Proc. Natl. Acad. Sci. USA.* **67:** 220–295.
39. Fredrikson, G., P. Stralfors, N. O. Nilsson, and P. Belfrage. 1981. Hormone-sensitive lipase of rat adipose tissue: purification and some properties. *J. Biol. Chem.* **256:** 6311–6320.
40. Turtle, J. R., and D. M. Kipnis. 1967. An adrenergic receptor mechanism for control of cyclic 3′,5′ adenosine monophosphate synthesis in tissues. *Biochem. Biophys. Res. Commun.* **28:** 797–802.
41. Exton, J. H. 1980. Mechanisms involved in alpha-adrenergic phenomena: role of calcium ions in actions of catecholamines in liver and other tissues. *Am. J. Physiol.* **238:** E3–E12.
42. Partington, C. R., M. W. Edwards, and J. W. Daly. 1980. Regulation of cyclic AMP formation in brain tissue by alpha-adrenergic receptors: requisite intermediacy of prostaglandins of the E series. *Proc. Natl. Acad. Sci. USA.* **77:** 3024–3028.
43. Jackisch, R., R. Brückner-Schmidt, and G. Hertting. 1981. Endogenously formed PGE-2 mediates the increase in cAMP accumulation in rabbit splenic fibroblasts following alpha-receptor stimulation. *Eur. J. Pharmacol.* **71:** 151–155.
44. Sabol, S. L., and M. Nirenberg. 1979. Regulation of adenylate cyclase of neuroblastoma x glioma hybrid cells by alpha-adrenergic receptors. I. Inhibition of adenylate cyclase mediated by alpha receptors. *J. Biol. Chem.* **254:** 1913–1920.
45. Kato, R., and T. Nakaki. 1983. Alpha-2 adrenoceptors beyond cAMP generation: islets of Langerhans and intestinal epithelium. *Trends Pharmacol. Sci.* **4:** 34–36.
46. Aktories, K., G. Schultz, and K. H. Jakobs. 1979. Inhibition of fat cell adenylate cyclase by prostaglandin E and epinephrine: requirement for GTP and sodium ions. *FEBS Lett.* **107:** 100–104.
47. Aktories, K., G. Schultz, and K. H. Jakobs. 1980. Regulation of adenylate cyclase activity in hamster adipocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **312:** 167–173.
48. Jakobs, K. H., K. Aktories, and G. Schultz. 1982. Acceleration of adipocyte adenylate cyclase turn-off reaction by inhibitory hormonal factors. *Proc. Natl. Acad. Sci. USA.* **79:** 1373–1377.
49. McMahon, K. K., and R. J. Schimmel. 1981. Evidence for alpha-adrenergic activation and inactivation of phosphorylase in hamster adipocytes. *Mol. Pharmacol.* **20:** 339–344.
50. Schimmel, R. J., K. K. McMahon, and R. Serio. 1981. Interactions between alpha-adrenergic agents, prostaglandin E1, nicotinic acid, and adenosine in regulation of lipolysis in hamster epididymal adipocytes. *Mol. Pharmacol.* **19:** 248–255.
51. García-Sáinz, J. A., B. B. Hoffman, S-Y. Li, R. J. Lefkowitz, and J. N. Fain. 1980. Role of alpha-1 adrenoceptors in the turnover of phosphatidylinositol and of alpha-2 adrenoceptors in the regulation of cyclic AMP accumulation in hamster adipocytes. *Life Sci.* **27:** 953–961.
52. Kather, H., and B. Simon. 1981. Adrenoceptor of the alpha-2 subtype mediating inhibition of the human fat cell adenylate cyclase. *Eur. J. Clin. Invest.* **11:** 111–114.
53. Burns, T. W., P. E. Langley, B. E. Terry, D. B. Bylund, B. B. Hoffman, M. D. Tharp, R. J. Lefkowitz, J. A. García-Sáinz, and J. N. Fain. 1981. Pharmacological characterization of adrenergic receptors in human adipocytes. *J. Clin. Invest.* **67:** 467–475.
54. Lafontan, M., and M. Berlan. 1980. Evidence for the alpha-2 nature of the alpha-adrenergic receptor inhibiting lipolysis in human fat cells. *Eur. J. Pharmacol.* **66:** 87–93.
55. Wright, E. E., and E. R. Simpson. 1981. Inhibition of the lipolytic action of beta-adrenergic agonists in human adipocytes by alpha-adrenergic agonists. *J. Lipid Res.* **22:** 1265–1270.
56. Yamazaki, S., T. Katada, and M. Ui. 1982. Alpha-2-adrenergic inhibition of insulin secretion via interference with cyclic AMP generation in rat pancreatic islets. *Mol. Pharmacol.* **21:** 648–653.

57. Rabinovitch, A., E. Cerasi, and G. W. G. Sharp. 1978. Adenosine 3',5'-monophosphate-dependent and -independent inhibitory effects of epinephrine on insulin release in rat pancreatic islets. *Endocrinology.* **102:** 1733–1740.
58. Jakobs, K. H., and G. Schultz. 1979. Different inhibitory effect of adrenaline on platelet adenylate cyclase in the presence of GTP plus cholera toxin and of stable GTP analogues. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **310:** 121–127.
59. Aktories, K., and K. H. Jakobs. 1981. Epinephrine inhibits adenylate cyclase and stimulates a GTPase in human platelet membranes via alpha-adrenoceptors. *FEBS Lett.* **130:** 235–238.
60. Cassel, D., and Z. Selinger. 1977. Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site. *Proc. Natl. Acad. Sci. USA.* **74:** 3307–3311.
61. Aktories, K., G. Schultz, and K. H. Jakobs. 1982. Stimulation of low K_m GTPase by inhibitors of adipocyte adenylate cyclase. *Mol. Pharmacol.* **21:** 336–342.
62. Cooper, D. M. F., W. Schlegel, M. C. Lin and M. Rodbell. 1979. The fat cell adenylate cyclase system. Characterization and manipulation of its bimodel regulation by GTP. *J. Biol. Chem.* **254:** 8927–8931.
63. Schlegel, W., D. M. F. Cooper, and M. Rodbell. 1980. Inhibition and activation of fat cell adenylate cyclase by GTP is mediated by structures of different size. *Arch. Biochem. Biophys.* **201:** 678–682.
64. Aktories, K., G. Schultz, and K. H. Jakobs. 1981. Sodium amplifies adenosine receptor-mediated inhibition of adipocyte adenylate cyclase. *Eur. J. Pharmacol.* **71:** 157–160.
65. García-Sáinz, J. A., S-Y. Li, and J. N. Fain. 1981. Alpha-2-adrenergic amines, adenosine, and prostaglandins inhibit lipolysis and cyclic AMP accumulation in hamster adipocytes in the absence of extracellular sodium. *Life Sci.* **28:** 401–406.
66. Burns, T. W., P. E. Langley, B. E. Terry, D. B. Bylund, and L. R. Forte. 1982. Alpha$_2$-adrenergic activation inhibits forskolin-stimulated adenylate cyclase activity and lipolysis in human adipocytes. *Life Sci.* **31:** 815–821.
67. García-Sáinz, J. A. 1981. Decreased sensitivity to alpha-2 adrenergic amines, adenosine and prostaglandins in white fat cells from hamsters treated with pertussis vaccine. *FEBS Lett.* **126:** 306–308.
68. Hazeki, O., and M. Ui. 1981. Modification by islet-activating protein of receptor-mediated regulation of cyclic AMP accumulation in isolated rat heart cells. *J. Biol. Chem.* **256:** 2856–2862.
69. Katada, T., and M. Ui. 1981. Islet-activating protein, a modifier of receptor-mediated regulation of rat islet adenylate cyclase. *J. Biol. Chem.* **256:** 8310–8317.
70. Katada, T., and M. Ui. 1982. Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of membrane protein. *Proc. Natl. Acad. Sci. USA.* **79:** 3129–3133.
71. Katada, K., and M. Ui. 1982. ADP-ribosylation of the specific membrane protein of C6 cells by islet-activating protein associated with modification of adenylate cyclase activity. *J. Biol. Chem.* **257:** 7210–7216.
72. Fain, J. N., and C. O'Donnell. 1983. Reversal of the inhibitory effect of Gpp(NH)p on adipocyte adenylate cyclase activity by pertussis toxin. *Federation Proc.* **42:** 900(Abstract).
73. Tabachnick, I. I. A., and A. Gulbenkian. 1969. Adrenergic changes due to pertussis: insulin, glucose, and free fatty acids. *Eur. J. Pharmacol.* **7:** 186–195.
74. Gulbenkian, A., L. Schobert, C. Nixon, and I. I. A. Tabachnick. 1968. Metabolic effects of pertussis sensitization in mice and rats. *Endocrinology.* **83:** 885–892.
75. Fain, J. N., and P. B. Wieser. 1975. Effects of adenosine deaminase on cyclic adenosine monophosphate accumulation, lipolysis, and glucose metabolism of fat cells. *J. Biol. Chem.* **250:** 1027–1034.
76. Fain, J. N. 1973. Inhibition of adenosine cyclic 3',5'-monophosphate accumulation in fat cells by adenosine, N^6(phenylisopropyl)adenosine and related compounds. *Mol. Pharmacol.* **9:** 595–604.
77. Schwabe, U., R. Ebert, and H. C. Erbler. 1973. Adenosine release from isolated fat cells and its significance for the effects of hormones on cyclic 3',5'-AMP levels and lipolysis. *Arch. Exp. Pathol. Pharmakol.* **276:** 133–148.
78. Fain, J. N. 1979. Effect of lipolytic agents on adenosine and AMP formation by fat cells. *Biochim. Biophys. Acta.* **573:** 510–520.
79. Fredholm, B. B. 1978. Local regulation of lipolysis in adipose tissue by fatty acids, prostaglandins, and adenosine. *Med. Biol.* **59:** 249–261.
80. Fain, J. N., and C. C. Malbon. 1979. Regulation of adenylate cyclase by adenosine. *Mol. Cell. Biochem.* **25:** 143–169.
81. Villalobos Molina, R., and J. A. García-Sáinz. 1981. Effects of pertussis vaccine on the lipid metabolism of hamsters. *Life Sci.* **29:** 1021–1026.
82. Burns, T. W., P. E. Langley, B. E. Terry, and D. B. Bylund. 1982. Studies on desensitization of adrenergic receptors in human adipocytes. *Metabolism.* **31:** 288–293.
83. Burns, T. W., and P. E. Langley. 1970. Lipolysis by human adipose tissue: the role of cyclic 3',5'-adenosine monophosphate and adrenergic receptor sites. *J. Lab. Clin. Med.* **75:** 983–997.
84. Burns, T. W., P. E. Langley, and G. A. Robison. 1971. Adrenergic receptors and cyclic AMP in the regulation of human adipose tissue lipolysis. *Ann. NY Acad. Sci.* **185:** 115–128.
85. Carpene, C., M. Berlan, and M. Lafontan. 1983. Lack of functional antilipolytic alpha$_2$ adrenoceptor in rat fat cell: comparison with hamster adipocyte. *Comp. Biochem. Physiol.* **74C:** 41–45.
86. Lafontan, M., L. Dang Tran, and M. Berlan. 1979. Alpha adrenergic antilipolytic effect of adrenaline in human fat cells of the thigh: comparison with adrenaline responsiveness of different fat deposits. *Eur. J. Clin. Invest.* **9:** 261–266.
87. Pecquery, R., and Y. Giudicelli. 1980. Ontogenic development of alpha adrenergic receptors and responsiveness in white adipocytes. *Biochem. J.* **192:** 947–950.
88. Schimmel, R. J. 1976. Role of alpha and beta adrenergic receptors in control of glucose oxidation in hamster epididymal adipocytes. *Biochim. Biophys. Acta.* **428:** 379–387.
89. Kather, H., and F. Schroder. 1982. Adrenergic regulation of fat cell lipolysis in multiple symmetric lipomatosis. *Eur. J. Clin. Invest.* **12:** 471–474.
90. Kather, H., F. Schroder, and S. Bernd. 1982. Microdetermination of glycerol using bacterial NADH-linked luciferase. *Clin. Chim. Acta.* **120:** 295–300.
91. Pecquery, R., L. Malagrida, and Y. Giudicelli. 1980. Adipocyte adenylate cyclase and alpha- and beta-adren-

ergic receptors in one case of multiple symmetric lipomatosis. *Biomedicine.* **33:** 64–66.
92. Kather, H., and B. Simon. 1981. Adenylate cyclase of multiple lipomata. *Biomedicine.* **35:** 52–55.
93. Enzi, G., E. M. Inelman, A. Baritussio, P. Dorigo, M. Prosdocimi, and F. Mazzoleni. 1977. Multiple symmetric lipomatosis: a defect in adrenergic-stimulated lipolysis. *J. Clin. Invest.* **60:** 1221–1229.
94. Pecquery, R., L. Malagrida, and Y. Giudicelli. 1979. Direct biochemical evidence for the existence of alpha-adrenergic receptors in hamster white adipocyte membranes. *FEBS Lett.* **98:** 241–246.
95. García-Sáinz, J. A., I. Litosch, B. B. Hoffman, R. J. Lefkowitz, and J. N. Fain. 1981. Effect of thyroid status on alpha and beta catecholamine responsiveness of hamster adipocytes. *Biochim. Biophys. Acta.* **678:** 334–341.
96. Begin-Heick, N., P. A. Houlihan, and J. Sigouin. 1979. The identification of alpha adrenergic receptors in adipose tissue by [^{3}H]dihydroergocryptine binding. *Can. J. Biochem.* **57:** 1047–1049.
97. Pecquery, R., and Y. Giudicelli. 1980. Heterogeneity and subcellular localization of hamster adipocyte alpha adrenergic receptors. Evidence for alpha-1 and alpha-2 subtypes. *FEBS Lett.* **116:** 85–90.
98. Pecquery, R., and Y. Giudicelli. 1982. Catecholamine binding to the alpha-adrenergic receptors of hamster adipocytes. Evidence that guanine nucleotides regulate the binding to the alpha-2 receptors subtype. *Biochim. Biophys. Acta.* **714:** 14–25.
99. Giudicelli, Y., and R. Pecquery. 1981. Guanine nucleotides regulate specifically agonist binding affinity of the alpha-2 adrenergic receptor in white adipocytes. *FEBS Lett.* **130:** 301–304.
100. Berlan, M., and M. Lafontan. 1982. The $alpha_2$-adrenergic receptor of human fat cells: comparative study of $alpha_2$-adrenergic radio-ligand binding and biological response. *J. Physiol. (Paris).* **78:** 279–287.
101. Tharp, M. D., B. B. Hoffman, and R. J. Lefkowitz. 1981. Alpha-adrenergic receptors in human adipocyte membranes: direct identification by [^{3}H]yohimbine binding. *J. Clin. Endocrinol. Metab.* **52:** 709–714.
102. Gonzalez, J. L., C. Carpene, M. Berlan, and M. Lafontan. 1981. Discrepancy in the regulatory effects of sodium and guanine nucleotides on adrenaline and clonidine binding to alpha-2 adrenoceptors in human fat cell membranes. *Eur. J. Pharmacol.* **76:** 289–293.
103. Tolbert, M. E. M., F. R. Butcher, and J. N. Fain. 1973. Lack of correlation between catecholamine effects on cyclic adenosine 3′,5′-monophosphate and gluconeogenesis in isolated rat liver cells. *J. Biol. Chem.* **248:** 5686–5692.
104. Sherline, P., A. Lynch, and W. H. Glinsmann. 1972. Cyclic AMP and adrenergic receptor control of rat liver glycogen metabolism. *Endocrinology.* **91:** 680–690.
105. Birnbaum, M. J., and J. N. Fain. 1977. Activation of protein kinase and glycogen phosphorylase in isolated rat liver cells by glucagon and catecholamines. *J. Biol. Chem.* **252:** 528–535.
106. Malbon, C. C., H. R. Gilman, and J. N. Fain. 1980. Hormonal stimulation of cyclic AMP accumulation and glycogen phosphorylase activity in calcium-depleted hepatocytes from hypothyroid rats. *Biochem. J.* **188:** 593–599.
107. Pointer, R. H., F. R. Butcher, and J. N. Fain. 1976. Studies on the role of cyclic GMP and extracellular Ca^{2+} in the regulation of glycogenolysis in rat liver cells. *J. Biol. Chem.* **251:** 2987–2992.
108. Hutson, N. J., F. T. Brumley, F. D. Assimacopoulos, S. C. Harper, and J. H. Exton. 1976. Studies on the alpha adrenergic activation of hepatic glucose output. I. *J. Biol. Chem.* **251:** 5200–5208.
109. Cherrington, A. D., F. D. Assimacopoulos, S. C. Harper, J. D. Corbin, C. R. Park, and J. H. Exton. 1976. Studies on the alpha adrenergic activation of heptic glucose output. II. *J. Biol. Chem.* **251:** 5209–5218.
110. Keppens, S., J. R., Vandenheede, and H. De Wulf. 1977. On the role of calcium as second messenger in liver for the hormonally induced activation of glycogen phosphorylase. *Biochim. Biophys. Acta.* **496:** 448–457.
111. Exton, J. H. 1981. Molecular mechanisms involved in alpha adrenergic responses. *Mol. Cell. Endocrinol.* **23:** 233–264.
112. Fain, J. N. 1978. Hormones, Membranes, and Cyclic Nucleotides. 1978. Vol. 6A of the series Receptors and Recognition. P. Cuatrecasas and M. Greaves, editors. Chapman and Hall, London. 1–61.
113. Hems, D. A., and P. D. Whitton. 1980. Control of hepatic glycogenolysis. *Physiol. Rev.* **60:** 1–50.
114. Williamson, J. R., R. H. Cooper, and J. B. Hoek. 1981. Role of calcium in the hormonal regulation of liver metabolism. *Biochem. Biophys. Acta.* **639:** 243–295.
115. Tolbert, M. E. M., A. C. White, K. Aspry, J. Cutts, and J. N. Fain. 1980. Stimulation by vasopressin and alpha catecholamines of phosphatidylinositol formation in isolated rat liver parenchymal cells. *J. Biol. Chem.* **255:** 1938–1944.
116. Kneer, N. M., M. J. Wagner, and H. A. Lardy. 1979. Regulation by calcium of hormonal effects on gluconeogenesis. *J. Biol. Chem.* **254:** 12160–12168.
117. Aggerbeck, M., G. Guellaen, and J. Hanoune. 1980. Adrenergic receptor of the $alpha_1$-subtype mediates the activation of the glycogen phosphorylase in normal rat liver. *Biochem. Pharmacol.* **29:** 643–645.
118. Hoffman, B. B., T. Michell, M. D. Kilpatrick, R. J. Lefkowitz, M. E. M. Tolbert, H. Gilman, and J. N. Fain. 1980. Agonist versus antagonist binding to alpha adrenergic receptors. *Proc. Nat. Acad. Sci. USA.* **77:** 4569–4573.
119. García-Sáinz, J. A., and J. N. Fain. 1980. Effect of insulin, catecholamines and calcium ions on phospholipid metabolism in isolated white fat cells. *Biochem. J.* **186:** 781–789.
120. García-Sáinz, J. A., and J. N. Fain. 1980. Effect of adrenergic amines on phosphatidylinositol labeling and glycogen synthase activity in fat cells from euthyroid and hypothyroid rats. *Mol. Pharmacol.* **18:** 72–77.
121. Michell, R. H. 1975. Inositol phospholipids and cell surface receptor function. *Biochim. Biophys. Acta.* **415:** 81–147.
122. Prpic, V., P. F. Blackmore, and J. H. Exton. 1982. Phosphatidylinositol breakdown induced by vasopressin and epinephrine in hepatocytes is calcium-dependent. *J. Biol. Chem.* **257:** 11323–11331.
123. Michell, R. H., C. J. Kirk, L. M. Jones, C. P. Downes, and J. A. Creba. 1981. The stimulation of inositol lipid metabolism that accompanies calcium mobilization in stimulated cells: defined characteristics and unanswered questions. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **296:** 123–137.
124. Fain, J. N., S-H. Lin, P. Randazzo, S. Robinson, and M.

Wallace. 1983. Hormonal regulation of glycogen phosphorylase in rat hepatocytes: activation of phosphatidylinositol breakdown by vasopressin and alpha$_1$ catecholamines. *In* Isolation, Characterization, and Use of Hepatocytes. R. A. Harris and N. W. Cornell, editors. Elsevier Publishing Co., Amsterdam and New York. 411–418.

125. Lawrence, J. C., Jr., and J. Larner. 1977. Evidence for alpha-adrenergic activation of phosphorylase and inactivation of glycogen synthase in rat adipocytes. Effects of alpha and beta adrenergic agonists and antagonists on glycogen synthase and phosphorylase. *Mol. Pharmacol.* **13:** 1060–1075.
126. Lawrence, J. C., Jr., and J. Larner. 1978. Effects of insulin, methoxamine, and calcium on glycogen synthase in rat adipocytes. *Mol. Pharmacol.* **14:** 1079–1091.
127. Giudicelli, Y., N. R. Thotakura, D. Lacasa, R. Pecquery, and B. Agli. 1981. Direct binding studies do not support the existence of true alpha-1 adrenoceptors in rat white fat cells. *Biochem. Biophys. Res. Commun.* **100:** 621–628.
128. Khoo, J. C. 1976. Ca^{2+}-dependent activation of phosphorylase by phosphorylase kinase in adipose tissue. *Biochim. Biophys. Acta.* **422:** 87–97.
129. Pershadsingh, H. A., and J. M. McDonald, 1979. Direct addition of insulin inhibits a high affinity Ca^{2+}-ATPase in isolated adipocyte plasma membranes. *Nature.* **281:** 495–497.
130. Perry, M. C., and C. N. Hales. 1970. Factors affecting the permeability of isolated fat cells from the rat to [^{42}K]potassium and [^{36}Cl]chloride ions. *Biochem. J.* **117:** 615–621.
131. Stein, J. M., and C. N. Hales. 1972. Effect of adrenaline on [^{32}P] incorporation into rat fat cell phospholipids. *Biochem. J.* **128:** 531–541.
132. Kiechle, F. L., J. F. Strauss III, T. Tanaka, and L. Jarett. 1982. Phospholipids as possible chemical mediators of insulin action on pyruvate dehydrogenase. *Federation Proc.* **41:** 1082 (Abstract).
133. Farese, R. V., R. E. Larson, and M. A. Sabir. 1982. Insulin acutely increases phospholipids in the phosphatidate-inositide cycle in rat adipose tissue. *J. Biol. Chem.* **257:** 4042–4045.
134. Litosch, I., Y. Saito, and J. N. Fain. 1982. 5-HT-stimulated arachidonic acid release from labeled phosphatidylinositol in blowfly salivary glands. *Am. J. Physiol.* **243:** C222–C226.
135. Fain, J. N., and M. J. Berridge. 1979. Relationship between hormonal activation of phosphatidylinositol hydrolysis, fluid secretion, and calcium flux in the blowfly salivary gland. *Biochem. J.* **178:** 45–58.
136. Fain, J. N., and M. J. Berridge. 1979. Relationship between phosphatidylinositol synthesis and recovery of 5-hydroxytryptamine-responsive Ca^{2+} flux in blowfly salivary glands. *Biochem. J.* **180:** 655–661.
137. Lin, S-H., and J. N. Fain. 1981. Vasopressin and epinephrine stimulation of phosphatidylinositol breakdown in the plasma membrane of rat hepatocytes. *Life Sci.* **29:** 1905–1912.
138. Wallace, M. A., P. Randazzo, S-Y. Li, and J. N. Fain. 1982. Direct stimulation of phosphatidylinositol degradation by addition of vasopressin to purified rat liver plasma membranes. *Endocrinology.* **111:** 341–343.
139. Harrington, C. A., C. M. Davis, and J. Eichberg. 1982. Characterization of norepinephrine-stimulated breakdown of phosphatidylinositol in purified rat liver plasma membrane. Abstract of the Endocrine Society Meeting, # 271.
140. Blackmore, P. F., B. F. Hughes, E. A. Shuman, and J. H. Exton. 1983. Alpha adrenergic activation of phosphorylase in liver cells involves mobilization of intracellular calcium without influx of extracellular calcium. *J. Biol. Chem.* **257:** 190–197.
141. Fain, J. N. 1981. Catecholamine–thyroid hormone interactions in liver and adipose tissue. *Life Sci.* **28:** 1745–1754.
142. Rosenqvist, U., S. Efendic, B. Jereb, and J. Ostman. 1971. Influence of the hypothyroid state on lipolysis in human adipose tissue in vitro. *Acta Med. Scand.* **189:** 381–384.
143. Giudicelli, Y., D. Lacasa, and B. Agli. 1980. White fat cell alpha-adrenergic receptors and responsiveness in altered thyroid status. *Biochem. Biophys. Res. Commun.* **94:** 1113–1122.
144. Malbon, C. C., F. J. Moreno, R. J. Cabelli, and J. N. Fain. 1978. Fat cell adenylate cyclase and beta-adrenergic receptors in altered thyroid states. *J. Biol. Chem.* **253:** 671–678.
145. Malbon, C. C. 1980. The effects of thyroid status on the modulation of fat cell beta-adrenergic receptor agonist affinity by guanine nucleotides. *Mol. Pharmacol.* **18:** 193–198.
146. Peake, G. T., C. A. Birge, and W. H. Daughaday. 1973. Alterations of radioimmunoassayable growth hormone and prolactin during hypothyroidism. *Endocrinology.* **92:** 487–493.
147. Czech, M. P., C. C. Malbon, W. Gitomer, and P. F. Pilch. 1982. Effect of thyroid status on insulin action in rat adipocytes and skeletal muscle. *J. Clin. Invest.* **66:** 574–582.
148. Correze, C., R. Auclair, and J. Nunez. 1976. Cyclic nucleotide phosphodiesterases, insulin, and thyroid hormones. *Mol. Cell. Endocrinol.* **5:** 67–79.
149. Armstrong, K. J., J. E. Stouffer, R. G. Van Inwegen, W. J. Thompson, and G. A. Robison. 1974. Effects of thyroid hormone deficiency on cyclic adenosine 3′,5′-monophosphate and control of lipolysis in fat cells. *J. Biol. Chem.* **249:** 4226–4231.
150. Van Inwegen, R. G., G. A. Robison, W. J. Thompson, K. J. Armstrong, and J. E. Stouffer. 1975. Cyclic nucleotide phosphodiesterase and thyroid hormones. *J. Biol. Chem.* **250:** 2452–2456.
151. Schoenle, E., J. Zapf, and E. R. Froesch. 1981. In vivo control of insulin-sensitive phosphodiesterase in rat adipocytes by growth hormone and its parallelism to glucose transport. *Endocrinology.* **109:** 561–566.
152. Schoenle, E., J. Zapf, and E. R. Froesch. 1982. Glucose transport in adipocytes and its control by growth hormone in vivo. *Am. J. Physiol.* **242:** E368–E372.
153. Malbon, C. C., and D. M. Gill. 1979. ADP-ribosylation of membrane proteins and activation of adenylate cyclase by cholera toxin in fat cell ghosts from euthyroid and hypothyroid rats. *Biochim. Biophys. Acta.* **586:** 518–527.
154. Burns, T. W., P. A. Boyer, B. E. Terry, P. E. Langley, and G. A. Robison. 1979. The effect of fasting on the adrenergic receptor activity of human adipocytes. *J. Lab Clin. Med.* **94:** 387–394.
155. Berlan, M., C. Carpene, M. Lafontan, and L. Dang Tran. 1982. Alpha$_2$-adrenergic antilipolytic effect on

dog fat cells: incidence of obesity and adipose tissue localization. *Horm. Metabol. Res.* **14:** 257–260.
156. Arner, P., P. Engfeldt, and J. Östman. 1979. Relationship between lipolysis, cyclic AMP, and fat-cell size in human adipose tissue during fasting and in diabetes mellitus. *Metabolism.* **28:** 198–209.
157. Östman, J., P. Arner, P. Engfeldt, and L. Kager. 1979. Regional differences in the control of lipolysis in human adipose tissue. *Metabolism.* **28:** 1198–1205.
158. Kather, H., K. Zollig, B. Simon, and G. Schlierf. 1977. Human fat cell adenylate cyclase. Regional differences in adrenaline responsiveness and obesity. *Eur. J. Clin. Invest.* **7:** 595–597.
159. Curtis-Prior, P. B. 1975. Prostaglandins and obesity. *Lancet.* **i:** 897–899.
160. Giudicelli, Y., and R. Pecquery. 1978. Beta-adrenergic receptors and catecholamine-sensitive adenylate cyclase in rat fat cell membranes: influence of growth, cell size, and aging. *Eur. J. Biochem.* **90:** 413–419.
161. Bertrand, H. A., E. J. Masoro, and B. P. Yu. 1980. Maintenance of glucagon-promoted lipolysis in adipocytes by food restriction. *Endocrinology.* **107:** 591–595.
162. Yu, B. P., H. A. Bertrand, and E. J. Masoro. 1980. Nutrition-aging influence of catcholamine-promoted lipolysis. *Metabolism.* **29:** 438–444.
163. Dax, E. M., J. S. Partilla and R. I. Gregerman. 1981. Mechanisms of the age-related decrease of epinephrine-stimulated lipolysis in isolated rat adipocytes: beta-adrenergic receptor binding, adenylate cyclase activity, and cyclic AMP accumulation. *J. Lipid Res.* **22:** 934–943.
164. Jolly, S. R., Y. B. Lombardo, J. J. Lech, and L. A. Menahan. 1980. Effect of aging and cellularity on lipolysis in isolated mouse fat cells. *J. Lipid Res.* **21:** 44–52.
165. Giudicelli, Y., D. Lacasa, and B. Agli. 1982. Alterations induced by a prolonged fasting: opposite effects on the beta-adrenergic receptor-coupled adenylate cyclase system and on lipolysis in fat cells from rat. *Eur. J. Biochem.* **121:** 301–308.
166. Schimmel, R. J., R. Serio, and K. K. McMahon. 1980. Alpha-adrenergic inhibition of cyclic AMP accumulation in hamster adipocytes. *Biochim. Biophys. Acta.* **632:** 544–552.
167. Rodbell, M., H. M. J. Krans, S. L. Pohl, and L. Birnbaumer. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. IV. Effects of guanyl nucleotides on binding of ^{125}I glucagon. *J. Biol. Chem.* **246:** 1872–1876.
168. Rodbell, M., L. Birnbaumer, S. L. Pohl, and H. M. J. Krans. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. V. An obligatory role of guanyl nucleotides in glucagon action. *J. Biol. Chem.* **246:** 1877–1882.
169. Nedergaard, J., and O. Lindberg. 1982. The brown fat cell. *Int. Rev. Cytol.* **74:** 187–286.
170. Svoboda, P., J. Svartengren, M. Snochowski, J. Houstek, and B. Cannon. 1979. High number of high-affinity binding sites for (−)-[^{3}H]dihydroalprenolol on isolated hamster brown fat cells. A study of the beta-adrenergic receptors. *Eur. J. Biochem.* **102:** 203–210.
171. Svartengren, J., N. Mohell, and B. Cannon. 1980. Characterization of [^{3}H]dihydroergocryptine binding sites in brown adipose tissue. Evidence for the presence of alpha-adrenergic receptors. *Acta Chem. Scand. B.* **34:** 231–232.
172. Mohell, N., M. Wallace, and J. N. Fain. 1982. Adrenergic stimulation of phosphatidylinositol turnover in hamster brown fat cells: a comparison to other alpha$_1$ adrenergic effects. *Acta Physiol. Scand.* **Supp. 508:** 64.
173. Strunecka, A., L. Olivierusova, V. Kubista, and Z. Drahota. 1981. Effect of cold stress and norepinephrine on the turnover of phospholipids in brown adipose tissue of the golden hamster. *Physiol. Bohemoslov.* **30:** 307–313.
174. García-Sáinz, J. A., A. K. Hasler, and J. N. Fain. 1980. Alpha$_1$-adrenergic activation of phosphatidylinositol labeling in isolated brown fat cells. *Biochem. Pharmacol.* **29:** 3330–3333.
175. McMahon, K. K., and R. J. Schimmel. 1982. Apparent absence of alpha$_2$-adrenergic receptors from hamster brown adipocytes. *Life Sci.* **30:** 1185–1192.
176. Hittelman, K. J., R. Bertin, and R. W. Butcher. 1974. Cyclic AMP metabolism in brown adipocytes of hamsters exposed to different temperatures. *Biochim. Biophys. Acta.* **338:** 398–407.
177. Ikemoto, H., T. Hiroshige, and S. Itoh. 1967. Oxygen consumption of brown adipose tissue in normal and hypothyroid mice. *Jpn. J. Physiol.* **17:** 516–522.
178. Seydoux, J., J. P. Giacobino, and L. Girardier. 1982. Impaired metabolic response to nerve stimulation in brown adipose tissue of hypothyroid rats. *Mol. Cell. Endocrinol.* **25:** 213–226.
179. Sellers, E. A., and S. S. You. 1950. Role of thyroid in metabolic responses to a cold environment. *Am. J. Physiol.* **163:** 81–91.
180. Sellers, E. A., K. V. Flattery, and G. Steiner. 1974. Cold acclimation of hypothyroid rats. *Am. J. Physiol.* **226:** 290–294.
181. Sundin, U. 1981. GDP binding to rat brown fat mitochondria: effects of thyroxine at different ambient temperatures. *Am. J. Physiol.* **241:** C134–C139.
182. Triandafillou, J., C. Gwilliam, and J. Himms-Hagen. 1982. Role of thyroid hormone in cold-induced changes in rat brown adipose tissue mitochondria. *Can. J. Biochem.* **60:** 530–537.
183. Rothwell, N. J., and M. J. Stock. 1979. A role for brown adipose tissue in diet-induced thermogenesis. *Nature.* **281:** 31–35.
184. Himms-Hagen, J., J. Triandofillou, and C. Gwilliam. 1981. Brown adipose tissue of cafeteria-fed rats. *Am. J. Physiol.* **241:** E116–E120.
185. Sundin, U., and M. Nechad. 1983. Trophic response of rat brown fat by glucose feeding. Involvement of the sympathetic nervous system. *Am. J. Physiol.* **244:** C142–C149.