AGONIST-RECEPTOR INTERACTIONS INVOLVED WITH MOBILIZATION OF FREE FATTY ACIDS FROM ADIPOSE TISSUE

BY

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INTRODUCTION

Previous Work

The understanding of the morphology and physiology of adipose tissue has changed markedly in the last thirty years. The fat stores of adipose tissue are no longer thought of in the role of merely insulating the body against heat loss or in giving support to other tissues, but rather in a new metabolic role of maintaining the caloric homeostasis of the body.

The importance of this tissue was long overlooked because early histological preparations showed 95% of the adipose tissue to be a foam-like cluster of fat droplets and the remaining 5% to be connective tissue and blood vessels. Actually, only a very few capillaries could be sighted and there seemed to be a virtual absence of nerves. It seemed very likely that this tissue was metabolically inert, participating only in sluggish and passive reactions. The cytoplasm, constituting about 2% of the wet weight and later to be recognized as the metabolically active portion of the fat cell, was nearly invisible and its significance therefore obscured.

Until the late 1940's, adipose tissue remained secondary in importance to the liver as the predominant organ of metabolism of lipids. An increasing number of investigations did not conform to this opinion and research workers began to attribute a greater importance to adipose tissue. Rosenfeld (1) and Gierke (2) ascribed an important role to adipose tissue for the conversion of carbohydrates to fat and glycogen. Wassermann (3) concluded on the basis of histological and embryological studies that adipose tissue was unique in

the accumulation and mobilization of fat, and a clear demonstration by the work of Schoenheimer and Rittenberg (4) of a turnover of fat in the body indicated the involvement of fat in the dynamic energy processes of the body. Wertheimer and Shapiro (5), in a classical review, sought to stress the importance of adipose tissue as a metabolically active organ influenced by nutritional, nervous and endocrine factors. The conclusions expressed by these authors provided the impetus for an unprecedented interest in adipose tissue.

The accumulation of results obtained with both the light and electron microscopes confirmed the new concept of adipose tissue and substantiated the fact that triglyceride transformation and other biochemical reactions occur in the cytoplasm of the fat cell (6). The finer observations of the fat cell possible with the electron microscope have clearly demonstrated accumulation and mobilization of fat in adipose tissue and the acknowledged presence of mitochondria, ribonucleoprotein particles, endoplasmic reticulum or microsomes, liquid droplets and vesicles serves to emphasize that fat is an active metabolic tissue (7,8). Electron micrographs of differentiated white adipose cells revealed the existence of a large central lipid mass surrounded by a thin rim of cytoplasm. The cell is enclosed by a basement membrane and the nucleus is displaced to a peripheral position. Capillaries have been observed closely applied to the surface of fat cells and neural elements have been located in the vicinity of cells, but rarely present intracellularly (9).

Barrnett and Ball (10) initially observed invaginations in the surface of the fat cell membrane and vesicles in the cytoplasm of fat cells previously incubated with insulin. They suggested that fat

transport occurred through the formation of these pinocytotic vesicles.

A number of investigators have reported marked alterations of the fat cell under a variety of experimental conditions. Observation of fat pads from animals either acutely starved or on a chronic low food intake (7,11,12) indicated a decrease in the cell volume and an extensive loss of lipid stores, the latter, being nearly absent in some fat cells. In addition, a marked increase in vesicular formations occurs uniformly along the plasma membrane indicating augmented pinocytotic activity.

The demonstration by electron microscopy of glycogen accumulation in the cytoplasm of cells from diabetic rats treated with insulin or from refed, fasted rats supports the work of Fawcett (13) with the light microscope. The presence of these glycogen granules indicated the ability of the fat cell to incorporate carbohydrate during the synthesis of new fat.

The organelles, mitochondria and microsomes, are thought to be intimately involved with glyceride synthesis in adipose tissue. Microscopic examination of a cell-free preparation of rat adipose tissue capable of synthesizing glycerides from the added precursors, α-glycerophosphate and fatty acids was shown to contain mitochondria, microsomes and membrane-enclosed lipid droplets (14,15). The removal of the mitochondria and/or microsomes from this cell-free preparation markedly altered the esterification rate (16). The intracellular localization of newly synthesized lipid-C^{14} from glucose-u-C^{14} in free adipose cells was found in the mitochondria, microsomes, lipid droplets and the lipid mass or bulk lipid (17). It was postulated that these liquid droplets are the transport form of the glycerides from the sites of

synthesis to the sites of storage.

The mechanisms of morphological changes associated with lipolysis and lipogenesis in fat cells are not clearly defined at this time, however the modern concept suggests that adipose tissue is a two-compartmental system, (a) a large storage compartment with a slow turnover rate and (b) a smaller compartment possessing a rapid turnover rate. The location of the adipose tissue cell near capillaries and nervous innervation serves to supplement this concept of adipose tissue as an active center of energy metabolism.

A fundamental barrier to the understanding of adipose tissue metabolism and its relationship to the functions of the whole organism was the lack of knowledge of the mechanisms of fat mobilization and transportation to other tissues. Favarger (18) had postulated that free fatty acids might be the mobilized form of fat in the body. Free fatty acids (FFA), also known as unesterified fatty acids (UFA) or nonesterified fatty acids (NEFA) among others, were known to exist in small quantities in blood plasma and bound to serum albumin but had been dismissed as relatively insignificant in the mobilization process partly because of inadequate quantitative procedures. It was not until 1956 when Gordon and Cherkes (19), and Dole (20) independently reported fluctuation of plasma FFA levels with changes in the nutritional state of human subjects that the importance of this plasma fraction was realized. It was observed that feeding of carbohydrate or injection of insulin caused a fall in plasma FFA levels whereas fasting or injection of epinephrine caused a rise in plasma FFA. Thus, the FFA released in response to increased energy requirements of the body provide a readily transportable source of

energy to other tissues for oxidation or biosynthesis.

The FFA in the plasma have a concentration of about 0.6 moles per liter accounting for approximately 2-3% of the total plasma lipids. The ability to transport large amounts of FFA to tissue cells is mainly attributed to a rapid turnover rate in the plasma. The turnover rate of FFA in the bloodstream is about 20-50% per minute in dogs (21) and in man (22) and up to 100% per minute in the rat (23). Calculations based on the half-life of FFA, the plasma level of FFA and the plasma volume indicate that a 200-gram rat releases enough FFA to account for approximately 25% of the caloric requirements of the fasting state. In man, the flux of FFA through plasma during twenty-four hours can account for about 60% of the daily energy requirements.

When the energy reserves of adipose tissue are mobilized as FFA into the blood stream, they enter a physiological fatty acid transport cycle (24). These FFA are bound to albumin (25) and transported to other tissues to be oxidized or stored where the uptake of the FFA by the tissues is strongly influenced by the plasma concentration. Liver assimilates about 30% of the FFA presented to it, transforming them to triglycerides, phospholipids and cholesterol esters. At the same time, liver secretes lipoproteins containing triglycerides. The extent to which these lipoprotein triglycerides are utilized as an energy substrate is unknown, however, Bezman, Felts and Havel (26) have reported uptake of lipoprotein triglycerides into adipose tissue. The return of some of the lipoprotein to be redeposited in adipose tissue completes the cycle. The involvement of the FFA with liver is representative of a homeostatic state brought

into play when the mobilization of FFA exceeds the immediate energy requirements.

The two major fuels for oxygen consumption in the body are carbohydrates and lipids. With the exception of the brain, all peripheral tissues are suited for the uptake and the use of FFA for oxidative needs. The regulation and availability of the energy substrates, FFA and glucose, to tissues is dependent upon the nutritional state of the organism. The plasma levels of FFA are elevated during fasting (19,20), production of fear or discomfort (27,28), orthostasis (29), trauma (30) or exercise (31,32), which, in turn, are influenced by the glucose concentration in the blood.

The relationship of glucose and FFA is particularly evident during carbohydrate feeding or fasting. Elevated blood glucose concentrations depress plasma FFA and a decrease in blood glucose increases the plasma FFA. Injection of labeled FFA in man (22), rat (33) and dog (34) followed by the recovery of expired CO_2 and measurement of $C^{14}O_2$ verified the fact that the plasma FFA are utilized as a major energy substrate in food deprivation. Fasting subjects expired significantly more $C^{14}O_2$ than the carbohydrate-fed subjects. Respiratory quotient values of 0.7 during fasting and 1.0 after glucose feeding, determined in dogs, serves to emphasize that the availability of the energy substrates, FFA and glucose, in the bloodstream are dependent upon the nutritional state of the animal.

Adipose tissue is the primary source of the plasma FFA and the mobilization is dependent upon the intracellular FFA concentration in adipose tissue cells (6,35,36,37). The control of plasma FFA is dependent upon two major variables, the breakdown of triglycerides or lipolysis and the synthesis of triglycerides or esterification.

These processes are in dynamic equilibrium and mobilization of FFA is achieved by the alteration of lipolysis and/or esterification rates. The triglycerides stored in adipose tissue are hydrolyzed to FFA and glycerol by the action of lipases (38,39,40). The reaction is not reversible, however, since the end products are not the immediate precursors for the esterification process. The FFA must first be activated to form fatty acyl coenzyme A derivatives which combine with α-glycerophosphate (not glycerol) initiating the synthesis of triglycerides. The intracellular free fatty acids, then, may be re-esterified to triglycerides, released to the plasma or oxidized by adipose tissue as an energy source.

The suppression of plasma FFA by glucose feeding is a result of an increased synthesis of triglycerides. Leboeuf et al. (41) established that the rate of esterification depends upon the amount of α-glycerophosphate available in adipose tissue and that glucose is an excellent precursor of this obligatory substrate for esterification. The adipose cell, therefore, is a site of carbohydrate and fat metabolism and the output of FFA is modulated by the metabolism of glucose.

The influence of the autonomic nervous system, and in particular the sympathetic nervous system, has an important role in lipid metabolism and transport. Dole (20) and Gordon and Cherkes (19), demonstrated elevation of plasma FFA in human subjects following the injection of the sympathetic hormone, epinephrine. White and Engel (42) and Gordon and Cherkes (43) demonstrated the release of FFA by epinephrine with epididymal adipose tissue, in vitro. The hormones, norepinephrine and epinephrine, were found to be equally

active and adipose tissue has shown specificity for the physiological isomer, *in vitro* (44). The intact sympathetic system is necessary for the mobilization of energy substrates in a stressful environment. This is illustrated by the fact that a large number of sympathetic blocking agents are capable of depressing lipid mobilization induced by the catecholamines or the sympathetic nervous system hormones and certain stress procedures (45). Havel and Goldfien (46) proposed that the sympathetic nervous system exerts a tonic action on the mobilization of fatty acids from adipose tissue. The ganglionic blocking agents, hexamethonium and trimethaphan, have decreased basal plasma FFA levels in both dog and man. Furthermore, the adrenergic blocking agents, phentolamine, dibenzyline, ergotamine, dibenamine, dichloroisoproterenol and nethalide have been shown to inhibit the release of FFA induced by catecholamines from adipose tissue, *in vitro*. The depression of sympathetic activity *in vivo* has been less successful because most of the antagonists found to be active *in vitro* acutely raise plasma FFA levels when administered *in vivo*.

The measurement of norepinephrine content in adipose tissue was the first direct evidence supporting the concept that the mobilization of FFA was under the influence of the sympathetic nervous system. Paoletti *et al.* (47) found significant amounts of norepinephrine, but practically no epinephrine. The norepinephrine content of white epididymal fat tissue has been reported by several groups to be in the range of 0.05-0.12 micrograms per gram of tissue (48,49). When expressed in terms of micrograms per milligram protein, this concentration is similar to that of norepinephrine in the heart and brain.

It is now generally agreed that norepinephrine in adipose tissue

is contained in storage sites associated with nervous tissue. The treatment of rats with reserpine or syrosingopine, alkaloids known to deplete catecholamine stores in other body tissues, also depletes adipose tissue (47,50). Stock and Westermann (50) reported that syrosingopine depleted adipose tissue and heart at the same rate, providing evidence for the similarity of the storage of norepinephrine in the two tissues. An increase in norepinephrine content in adipose tissue was obtained by treatment of the animals with a monoamine oxidase inhibitor, nialamide, and a parallel elevation of norepinephrine occurred in the tissues of the heart and brain.

The mobilization of FFA by the stimulation of adrenergic nerves supplying this tissue firmly established the physiological role of norepinephrine in adipose tissue. Correll (51,52) electrically stimulated nerves leading to isolated adipose tissue and measured the increased production of FFA. Starvation, known to sensitize adipose tissue to catecholamines, increased the response to stimulation two-fold as compared to the response of fed rats. Sympathectomy, timed to allow nerve degeneration, or the presence of the antagonist, dibenamine, markedly lowered the output of FFA after electrical stimulation. These studies established that adipose tissue is an effector organ capable of functioning under the neural influence of sympathetic origin.

The peripheral catecholamines play an important role in the mobilization of energy substrates via the sympathetic nervous system. The release of FFA and glucose as energy substrates in response to cold stress has been studied for the elucidation of this role of the sympathetic nervous system (53,54). A normal rat, placed in a cold room at 4°C., maintains thermal homeostasis by piloerection, vasocon-

striction and the mobilization of the energy substrates, FFA and glucose. A rat, depleted of peripheral stores of catecholamines by chemical sympathectomy and exposed to cold, is unable to mobilize the substrates and eventually dies. If the chemically sympathectomized rat has been pretreated with epinephrine prior to cold exposure, the animals again show thermal homeostatis, thus the administration of epinephrine restored the normal response to cold stress.

The sympathetic nervous system controls the output of FFA resulting from cold stimulus through the activation of an adipose tissue lipase, presumably by the release of norepinephrine at the terminal nerve endings in this tissue. Maickel et al. (55) were able to correlate the plasma FFA concentrations with lipase activity in adipose tissue. Elevation of plasma FFA and activation of lipase were observed in (a) an intact rat injected with catecholamines, (b) an intact rat injected with the ganglionic stimulant, 1,1-dimethyl-4-phenylpiperaxinium (DMPP) or (c) an intact rat exposed to cold at 4°C. No changes in FFA and lipase activity occurred in (a) an intact rat pretreated with the ganglionic blocking agent, chlorisondamine and exposure to cold, or (b) a chemically sympathectomized rat exposed to cold. The administration of DMPP to chemically sympathectomized animals did not change these responses. The activation of lipase and mobilization of FFA are therefore dependent upon the presence of a functional sympathetic nervous system.

In addition to the catecholamines mentioned above, several hormones have been reported to enhance the mobilization of FFA in an intact animal or with slices of adipose tissue, in vitro. Adipose tissue responds to these hormones by an increase in the activity of an

intracellular lipase, the production of glycerol, and the production of FFA (6,40). Adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), growth hormone (GH) and glucagon enhance the mobilization of FFA from rat adipose tissue, *in vitro* (57). Insulin, on the other hand, a major regulator of carbohydrate metabolism in the body, depresses the mobilization of FFA from adipose tissue, *in vivo* and *in vitro* (20,58,59,60).

Epinephrine, ACTH, glucagon and TSH have been shown to mobilize FFA by markedly stimulating the breakdown of triglycerides, and secondarily, to oppose the release of FFA by increasing the rate of esterification. In adipose tissue, there is an increase in incorporation of C^{14} from glucose into glyceride-glycerol in the presence of these lipolytic hormones (6,41,61). Glycerol, a product of lipolysis in adipose tissue, is not reutilized and is released into the incubation medium. The measurement of glycerol is a quantitative index of lipolysis and the measurement of FFA is a net result of the rates of lipolysis and re-esterification. Vaughan and Steinberg (62) simultaneously measured the production of FFA and glycerol, finding an increase in the rates of lipolysis and esterification. A net release of FFA observed with these hormones was due to the noticeable increase in the turnover rate of the triglycerides.

The lipolytic actions of norepinephrine and epinephrine are attributed to the activation of a hormone-sensitive lipase and two assay procedures have been used to measure the lipolytic activity of adipose tissue following the administration of hormones. The first involved the assay of lipolytic activity in crude or centrifuged homogenates of adipose tissue utilizing artificial triglyceride

substrates (38,63), and secondly, by a measurement of glycerol production (41,61). These experimental approaches have clearly established that the action of epinephrine is mediated by an increase in the rate of lipolysis.

Hormone-sensitive lipase has been studied in detail in the epididymal fat pad and differentiated from other intracellular lipases by studies in homogenates (38,39,40,64,65). This lipase system has been isolated in a subcellular fraction sedimenting out with microsomes. Furthermore, these studies have distinguished two lipase activities associated with lipolysis in adipose tissue, a monoglyceride lipase showing considerable activity on lower glycerides and a lipase active on triglycerides. The triglyceride-splitting lipase is particularly responsive to the hormones ACTH, TSH, glucagon, norepinephrine and epinephrine. Until appropriate fractionation procedures are developed, it is impossible to delegate the lipase activity to one or more specific enzymes.

A partial purification and separation of lipases based on substrate specificity, pH optimum, hormone and inhibitor characteristics has been reported (66). Usually 60-70% of the total lipase activity was found associated with the fat cake after centrifugation of adipose tissue homogenates prepared in 0.15 M KCl or 0.25 M sucrose. Lipase measurements prepared from the lower portion of the fat cake exhibited lipolytic activities of the monoglyceride and triglyceride-splitting lipases. Vaughan et al. (40) separated lipase activity from fresh rat and rabbit homogenates by preparing acetone powders and found a large fraction of monoglyceride lipase activity with only negligible activity on diolein or triglyceride substrates. Strand et al. (39) extracted 95-99% of esterified fatty acids from homogenates of adipose

tissue with peroxide-free ethyl ether leaving appreciable lipase activity on exogenously added mono-, di-, and triglyceride substrates. The use of suitable assay conditions of these preparations employing exogenous glycerides has permitted the differentiation of at least two intracellular lipases. Norepinephrine and epinephrine produced a marked increase in the activation of the triglyceride lipase as compared to the monoglyceride lipolytic activity. The triglyceride-splitting lipase exhibited a pH optimum at 7.5, responded to the hormonal stimulation and was inhibited by isopropanol. The monoglyceride lipase exhibited activity against mono- and diglyceride exogenous substrates, possessed a pH optimum of about 8.0 and was less responsive to the addition of norepinephrine. Based on presently available data, the triglyceride lipase appears to be similar, if not identical to the hormone-sensitive lipase in the intact tissue.

Interest in the effects of catecholamines on lipid metabolism was preceded by the studies of the catecholamines in carbohydrate metabolism. Sutherland and Rall (67,68) had extensively explored the glycogenolytic mechanism of the catecholamines and other hormones in liver preparations. They found that epinephrine appears to interact with adenyl cyclase, a cyclising enzyme which catalyzes the formation of adenosine 3',5'-monophosphate (3',5'-AMP) from adenosine triphosphate in liver homogenates. The cyclic nucleotide, 3',5'-AMP, stimulates the activity of dephospho-phosphorylase kinase which, in turn, catalyzes the formation of an active phosphorylase. The degradation of the 3',5'-AMP to yield 5'-AMP and orthophosphate is catalyzed by the enzyme phosphodiesterase. The methyl xanthines, caffeine and theophylline, have been shown to have an inhibitory effect upon

phosphodiesterase and thus, when added to the system, prevent the metabolic degradation of 3',5'-AMP. The distribution of the phosphodiesterase and adenyl cyclase enzymes is widespread and includes adipose tissue.

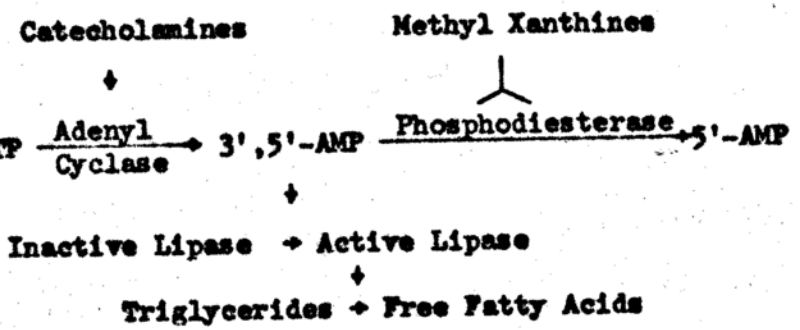
Several hormones have been shown to elevate the concentration of the cyclic nucleotide in adipose tissue. These include the catecholamines, glucagon, vasopressin and ACTH (69,70). Vaughan (71) reported the enhancement of phosphorylase activity and the subsequent release of FFA by several of the hormones. In addition, she found that serotonin increased the 3',5'-AMP levels and phosphorylase activity, but produced no mobilization of FFA. This apparent discrepancy was resolved when it was found that either pretreatment of adipose tissue with a monoamine oxidase inhibitor (72) or increasing the concentration of serotonin (66) elevated the release of FFA to a measureable extent.

The participation of 3',5'-AMP in lipolysis was indirectly substantiated by results from studies with caffeine and theophylline. Caffeine and theophylline potentiate hormone-induced release of FFA when added to adipose tissue, *in vitro* (62,64,73,74). Hynie *et al.* (74) found that theophylline evoked a lipolytic response that increased with the concentration of xanthine and reached a maximum effect three times that of norepinephrine. It is apparent from these and other studies that the rate of lipolysis is controlled by the steady state concentration of the cyclic nucleotide, which is further regulated by the relative activities of adenyl cyclase and phosphodiesterase.

The addition of the cyclic nucleotide to adipose tissue established a cause-effect relationship for the action of epinephrine.

Early attempts to demonstrate the lipolytic action of 3',5'-AMP on the fat pad *in vitro* failed (71), in part due to the poor penetrability and high susceptibility to inactivation of 3',5'-AMP. This finding led to the synthesis of less labile derivatives. The analogue, N^6-2'-O-dibutyryl cyclic 3',5'-AMP, was shown to stimulate lipolysis in incubated fat pads and also in isolated fat cells (56,75). Later Rodbell (76) and Maickel *et al.* (77) reported the stimulation of lipolysis by 3',5'-AMP in isolated fat cells, *in vitro*. The more stable analogue was at least ten times as active as the cyclic nucleotide in this preparation.

The diagram below represents a summary of the probable interrelationships of adenyl cyclase, 3',5'-AMP, lipase and the catecholamines:



The catecholamines initiate the mobilization of FFA by interaction with the adenyl cyclase system which in turn elevates the level of 3',5'-AMP. The cyclic nucleotide converts an inactive lipase to an active lipase which then increases the rate of lipolysis. The presence of a methyl xanthine to inhibit the metabolic degradation of the cyclic nucleotide facilitates the mobilization of FFA.

For many years, investigators distinguished between the inhibitory and excitatory effects of sympathomimetic amines as established

by Dale (78). This classification was never satisfactory and Ahlquist (79,80,81) in 1948 differentiated two types of adrenergic receptors, called alpha and beta, on the basis of responses to a variety of sympathomimetic amines on peripheral organs. Today, this concept has been modified to include antagonists of the alpha and beta receptors. In general, the alpha receptors are more sensitive to norepinephrine and epinephrine than to isopropyl arterenol and are blocked by the antagonists, phentolamine and dibenzyline. The beta receptors are more sensitive to isopropyl arterenol and are blocked by the antagonists, pronethalol and dichloroisoproterenol. Although exceptions to this classification have led to some criticism, the use of the terms alpha and beta receptors provides a useful frame of reference in the discussion of adrenergic effects.

The metabolic effects of the catecholamines, glycogenolysis and lipolysis, do not conform to the pattern of either a pure alpha or a pure beta receptor. The attempts to classify the fat mobilizing actions of the catecholamines has met with numerous difficulties and the effects have been attributed to alpha (82) or beta (83) adrenergic receptors, or to a yet unclassified type of receptor (84,85).

The adrenergic adipose tissue system responds more favorably to beta agonists. Numerous investigations have reported that the three main catecholamines, isopropyl arterenol (86,87,88,89), norepinephrine (89,90,46,44) and epinephrine (89,44,19,20,43) are very active in the mobilization of FFA, <u>in vivo</u> and <u>in vitro</u>. The beta agonist, isopropyl arterenol has been reported as the most potent of the three catecholamines, <u>in vitro</u> (89,91,92). An increased potency in a

homologous series of catecholamines and oxedrines(2-amino-1-p-hydroxyphenylethanol derivatives) with progressively larger N-alkyl substituents has substantiated the tendency of the receptor in adipose tissue to respond more favorably to the beta agonists, since these results are characteristic of the beta adrenergic receptor (93). In a recent study with hamster adipose tissue, the compounds methoxamine, mephenteramine, phenylpropanolamine and hydroxyamphetamine, all containing a strong alpha component of action, were much less active than the catecholamines (94). Furthermore, the pure alpha agonist, phenylephrine, was found to be nearly devoid of lipid mobilizing activity, in vivo (95) and in vitro (91,92).

The beta antagonists exhibit a greater degree of effectiveness and specificity than the alpha antagonists in the inhibition of lipid mobilization. Studies have reported that several adrenergic blocking agents inhibit the catecholamine-induced rise of FFA from adipose tissue, in vivo and in vitro. Although the alpha antagonists, dibenzyline (96,97), dibenamine (46,96), phentolamine (84,96,98) and ergotamine (99) are effective on catecholamine-induced FFA mobilization, the beta antagonists, dichloroisoproterenol (84,87,100, 103,104), pronethalol (83,101,103,104,105) and propanalol (102,104) are capable of blocking the mobilization by the catecholamines, both in vivo and in vitro. The inhibition by alpha antagonists is regarded as nonspecific and noncompetitive whereas the beta antagonists exhibit a competitive inhibition in the presence of various agonists. The effectiveness of both types of antagonists has produced conflicting views concerning the character of the adrenergic receptor.

The complexity of the classification has been increased by

recent developments. Many of the alpha and beta antagonists including phentolamine, dibenzyline, dichloroisoproterenol and pronethalol were found to be ineffective against the elevation of FFA during fasting (83,87,99,106,107). N-isopropylmethoxamine and butoxamine, which are classified neither as alpha nor beta antagonists, effectively blocked the elevation of FFA induced by fasting and catecholamines (108,109), but behaved like beta antagonists, in vitro (110). The adrenergic mechanism for the mobilization of FFA appeared to be involved with more than a one receptor system.

Stock and Westermann (110) have classified two sites of action for agonists and antagonists in adipose tissue. The beta antagonists inhibit the formation of the cyclic nucleotide (3',5'-AMP) whereas the alpha antagonists inhibit the action of the cyclic nucleotide. The beta antagonists, Ko 592, d-inpea (1-(p-nitrophenyl)-2-isopropylaminoethanol), and l-inpea competitively blocked norepinephrine-induced lipolysis and blocked the lipolysis of ACTH noncompetitively. In contrast, the alpha antagonist, phentolamine, was shown to inhibit the actions of norepinephrine noncompetitively, indicating that the sites of action are different. Maickel et al. (111) supported this relationship when they demonstrated that dichloroisoproterenol, in concentrations which effectively block the formation of the cyclic nucleotide by norepinephrine, failed to block the lipolysis of theophylline. Phentolamine exhibited a marked inhibition against theophylline-induced lipolysis. Coupled with results of other (75, 112), the actions of the beta antagonists are apparently mediated by an inhibition of the adenyl cyclase system while the alpha antagonists inhibit the action of the cyclic nucleotide prior to lipase activation.

These data suggest that adrenergic mobilization behaves like a beta receptor and the activation of adenyl cyclase followed by the formation of cyclic 3',5'-AMP and lipase activation is the probable mechanism of adrenergic agonists in adipose tissue.

Present Studies

The motivation for this research stems from the importance of FFA as a primary energy source in the body and as a contributing factor in certain disease states. Changes in the normal rate of lipid mobilization have been associated with obesity (113,114), lipomas (116), diabetes mellitus (115), cardiovascular diseases (119), pheochromocytoma (117) and hyperthyroidism (117,118). To alleviate or correct these metabolic abnormalities associated with lipid metabolism, widespread interest has been placed upon studies designed to elucidate the controlling mechanisms of FFA mobilization from adipose tissue.

An increasing amount of evidence indicates that the sympathetic nervous system may have an important role in the mobilization of lipids from adipose tissue depots. It has been shown that stimulation of sympathetic nervous supply to the epididymal fat pad results in a mobilization of FFA (51,52) and that the injection of epinephrine or norepinephrine increases plasma FFA levels (19,20,46). The addition of these catecholamines to an incubation medium containing adipose tissue slices results in a conversion of inactive lipase to an active lipase (40,56,64), which in turn leads to an increased mobilization of FFA. Based on the lipolytic activity possessed by the catechol-

amines, interest was directed towards the effects of other phenethylamines in adipose tissue.

Although many studies have reported the effects of catecholamines on lipid metabolism, few have defined the structure-activity relationships involved with the interaction of various phenethylamines and the adrenergic-adipose tissue receptor system. The following study presents information concerning the chemical specificity of agonists, and in particular, the substituted phenethylamines with the rat epididymal fat tissue, in vitro. This knowledge is deemed important for the future development of therapeutic agonists and antagonists of FFA mobilization and for an increased understanding of agonist-receptor interaction in this tissue.

EXPERIMENTAL

Materials

Chemicals. The chemicals* employed in this study and their sources are as follows: β-phenylethylamine, tyramine hydrochloride, DL-metanephrine hydrochloride, dopamine hydrochloride, l-phenylephrine hydrochloride, phenylpropanolamine hydrochloride, isopropyl arterenol hydrochloride, and DL-3-methoxy-4-hydroxymandelic acid (Mann Research Laboratories); 3,4-dihydroxymandelic acid, 2-isopropylaminoethanol, adrenalone hydrochloride, octopamine hydrochloride, N-α-methylbenzylmonoethanolamine, and d-amphetamine sulfate (K. & K. Laboratories); l-nordefrin, l-ethylnorepinephrine d-bitartrate monohydrate, l-epinephrine bitartrate (Sterling Winthrop Research Institute); metaproterenol sulfate (Geigy Pharmaceuticals); Nile Blue A indicator (Chicago Apparatus); l-norepinephrine bitartrate, norepinephrine, and bovine albumin, fraction V (Nutritional Biochemical Corp.); norphenylephrine hydrochloride, and N-benzylethanolamine (Aldrich Chemicals Company); homarylamine hydrochloride, phenisonone hydrobromide, metaraminol bitartrate, and ephedrine sulfate (Merck, Sharp and Dohme Laboratories); amidephrine methylsulfate and isoxuprine hydrochloride (Mead Johnson Company); nylidrin hydrochloride (U. S. Vitamin Corp.); and protochylol hydrochloride and N-methylepinephrine hydrochloride (Lakeside Laboratories); and reserpine phosphate (Ciba Laboratories). All concentrations expressed in this study refer to the free base or free acid.

*The author thanks colleagues in the pharmaceutical industry for generously supplying many of the chemicals used in this study.

Methods

Animals. White male Sprague-Dawley and Holtzman rats weighing between 200 and 250 grams were employed in all experiments. The rats were maintained in our animal quarters for one week prior to use. Non-fasted rats received Purina rat chow and water ad libitum, whereas fasted rats received access to water only. In studies with fasted animals, both pretreated and control rats were deprived of food for sixteen hours preceding the experiments.

In Vitro Free Fatty Acid Assay. Immediately after the animals were sacrificed by stunning and decapitation, the abdominal cavity was opened. The anterior one-third of each epididymal fat pad, devoid of large blood vessels, was excised, rinsed to remove contaminants, and transferred to a solution of freshly prepared Krebs-Ringer bicarbonate buffer, pH 7.4. Following the removal of fat pads from at least four rats per experiment, the tissue was minced with a small scissors to slices weighing approximately 5-10 mg.

Into each 30 ml. narrow neck incubation flask containing 9.0 ml. of 4% bovine albumin in Krebs-Ringer bicarbonate buffer, 600 mg. of tissue slices were added. All flasks prepared for the experiment were placed into a Dubnoff metabolic incubator maintained at 37°C. and oscillating at 110-120 revolutions per minute. Inhibitors were preincubated with the adipose tissue slices for 15 minutes prior to gassing, whereas catecholamines and other agonists were added in small volumes (0.05-0.40 ml.) and followed immediately by the gassing procedure.

Each incubation flask was then fitted with a rubber stopper

assembly through which were placed hypodermic needles (#20) fitted with PE 60 Intramedic polyethylene tubing to serve as vents for the gassing procedure and for use in sampling the media in the assay for free fatty acids (FFA). After all flasks were gassed with a 95% O_2/5% CO_2 atmosphere for ten minutes and each incubation system closed in this oxygen-rich environment, 0.5 ml. samples were withdrawn at 0, 20, 40, and 60 minutes and transferred to a centrifuge tube containing 5 ml. of an extraction mixture (composed of 40 parts isopropyl alcohol, 10 parts of heptane, and 1 part 1 N H_2SO_4). This system was divided into two phases by mixing into it an additional 2 ml. of heptane and 3.5 ml. of water, which separated rapidly after shaking to form a sharp interface between an upper heptane phase and a lower aqueous phase.

The titration solution is comprised of two phases prepared by the combination of a 2 ml. aliquot of the upper heptane phase and 1 ml. of an alcoholic Nile Blue A indicator solution. The procedure for the assay of FFA is a modification (120) of the method of Dole (20), employing the indicator Nile Blue A in the titration mixture. The Nile Blue A aliquot is prepared as a 1:10 dilution of an aqueous 20 mg. per cent stock solution with absolute ethyl alcohol. The stock solution of the dye was washed with three equivalent volumes of heptane to remove impurities. Nitrogen gas, delivered to the bottom of the titration solution through a capillary tube, was allowed to bubble freely to expel the CO_2 from the samples and keep the solution mixed during the titration with the titrant, approximately 0.02 N NaOH, being delivered with a microburet syringe. The alkali, prepared with CO_2-free water and stored in a tightly stoppered container, was

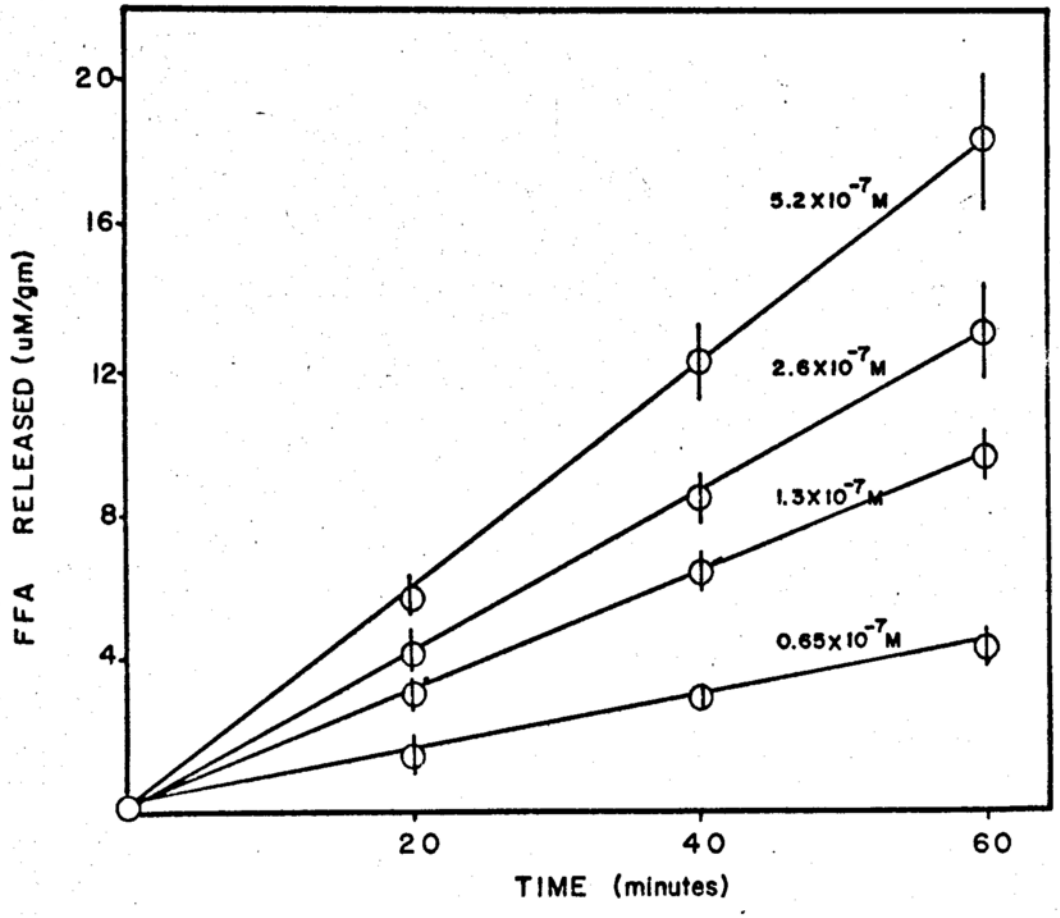
calibrated by extraction and titration of a known myristate standard (1 ueq./ml.) in heptane. A color change from blue to a persistent pink in the alcoholic phase was utilised as the endpoint of the titration.

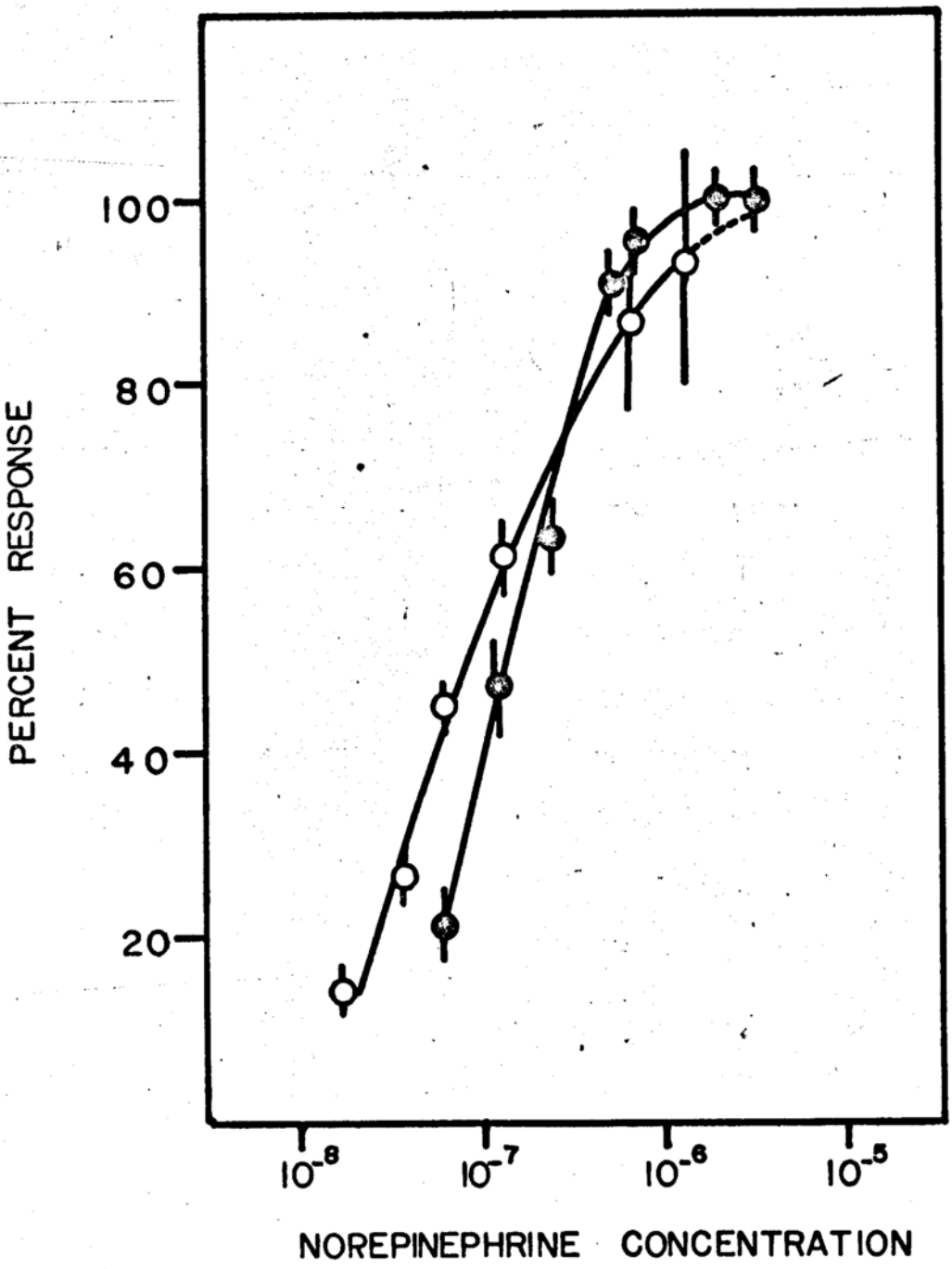
The determined concentrations of the FFA in each of the times sampled were plotted versus time and the rate of FFA release, expressed as μM./gm./hr. (micromoles FFA released per gram of tissue per hour of incubation) was calculated from the slope of the line obtained. Controls (no agonist present) were run in each experiment and the amount of FFA released in the absence of agonist was subtracted from the amount released in the presence of the agonist.

RESULTS AND DISCUSSION

Standard Dose-Response Relationship. Preliminary experimentation indicated that catecholamine-induced release of free fatty acids (FFA) was linear with respect to time for approximately two hours. It was observed, however, that the deviations from linearity were most frequent in the 90 to 120 minute period, and therefore the initial rate period of 0 to 60 minutes was chosen for all assays. The shorter time period also minimized errors due to degradation of catecholamines, as has been previously reported by Vaughan and Steinberg (62).

In Figure 1, the response of adipose tissue to various concentrations of norepinephrine is illustrated. It can be seen that the response rate is linear with respect to time for the 60 minute time period and as the concentration of norepinephrine is increased from 0.65×10^{-7} M to 5.2×10^{-7} M, there occurs an increase in the rate of FFA release. The maximum release rate found in the initial studies was found to be 18.0 micromoles per gram of tissue per hour (18 μM./gm./hr.) and was constant irrespective of the agonist employed. A more recent investigation of norepinephrine and other agonists in the adipose tissue system indicated a somewhat larger maximum release figure, calculated to be 22.2 μM./gm./hr. These maximum release rates were employed to calculate the per cent responses of the adipose tissue systems in all studies described herein. In Figure 2, the dose-response curves for norepinephrine corresponding to these two maximum rate figures are presented for purposes of comparison. Dose-


FFA RELEASED (uM/gm)
20
16
12
8
4
5.2X10⁻⁷M
2.6X10⁻⁷M
1.3X10⁻⁷M
0.65X10⁻⁷M
20
40
60
TIME (minutes)


PERCENT RESPONSE
100
80
60
40
20
10^{-8}
10^{-7}
10^{-6}
10^{-5}
NOREPINEPHRINE CONCENTRATION

response relationships similar to that shown in Figure 1 for norepinephrine were determined for each agonist and the rates of FFA release calculated as described previously. (See Methods)

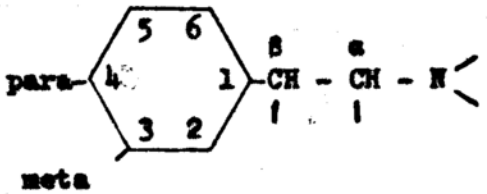
Dose-Response Relationships for Other Phenethylamine Agonists. The dose-response relationships of a number of structurally related phenethylamines were determined in an attempt to establish the degree of chemical specificity shown by the adipose tissue system. These relationships represent the resultant of the interaction of the agonists with complementary tissue components (receptors) in adipose tissue. In order to elicit a stimulus at least two properties are necessary for the drug molecule: (a) affinity or the ability to interact with the tissue receptors and (b) intrinsic activity (efficacy), the ability to interact in an effective manner to elicit the response (121). The agonist-adipose tissue receptor interactions involved with the mobilization of FFA conform to the relationship expressed by the mass action law or the Langmuir adsorption isotherm (122). Ariens (123) described an equation relating the relative response of a tissue to an agonist and the concentration of agonist employed which has been modified slightly to yield

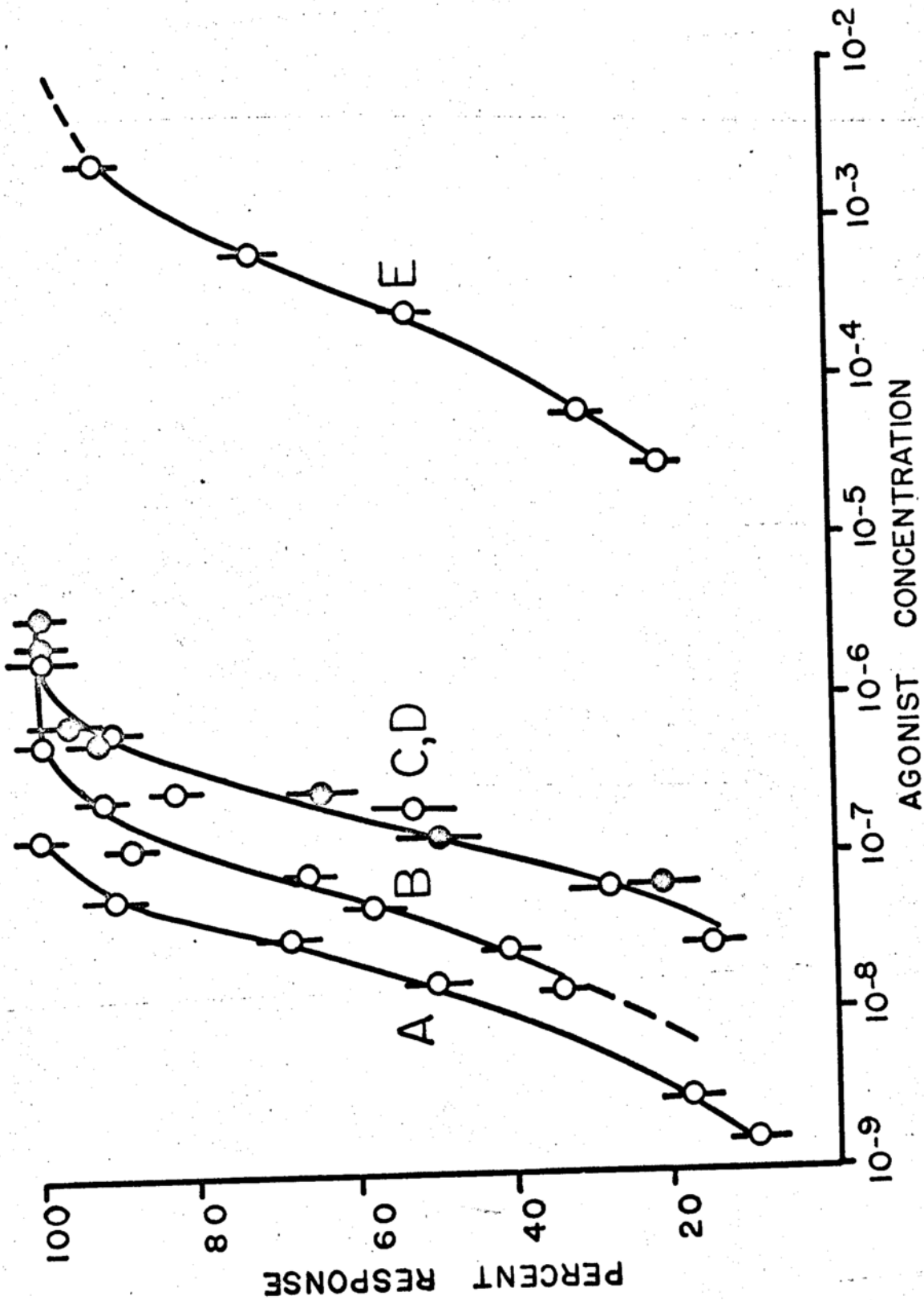
$$R = \frac{I(A)}{Ka + (A)}$$

where R is the response of the tissue measured as the rate of FFA release/gm. of tissue/hr., I is the intrinsic activity (maximal response obtained at infinite concentration of the agonist), A is the concentration of agonist employed, and Ka is the apparent dissociation constant of the agonist-receptor tissue complex. This mathematical

expression provides a means of calculating the affinity and intrinsic activity of the agonist. The affinity is proportional to the reciprocal of that concentration of the drug that gives a response equal to half the maximal response obtainable with the drug and the intrinsic activity is proportional to the maximal effect obtainable with the drug being employed. In these dose-response relationships, differences in affinity are shown by a shift of dose-response curves of the agonist along the log-dose axis, whereas a change in intrinsic activity results in an increase or decrease in the maximal effect obtainable with the compound. R may also be expressed (as has been done in these studies) as a per cent of the maximal response obtainable in the system.

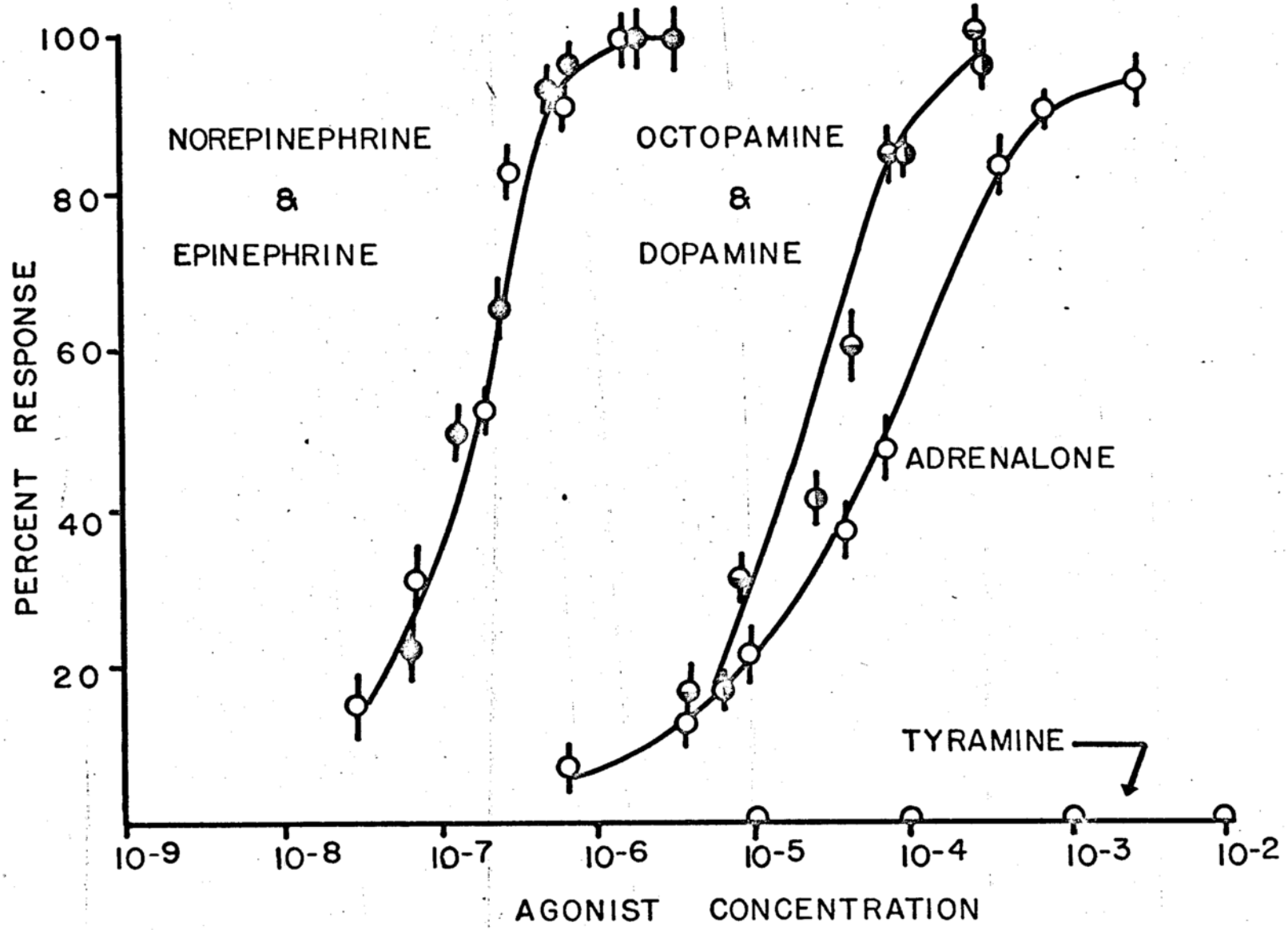
The dose-response relationships obtained for these agonists are presented in Figures 3-9. The structure of each compound is illustrated in Table II. It is apparent from these data that with the exception of the compounds phenylephrine (Figure 6) and phenylpropanolamine (Figure 7), all agonists were capable of producing a maximal release of FFA, thus, the agonists possess identical intrinsic activities. These agonists, however, differ in their relative affinities as shown by shifts in the dose-response curve. Structural modifications of the catecholamine molecule have produced marked changes in FFA mobilizing ability of agonists in adipose tissue slices and are discussed with particular reference to the positions as designated below:




PERCENT RESPONSE
100
80
60
40
20
10-9
10-8
10-7
10-6
10-5
10-4
10-3
10-2
AGONIST CONCENTRATION
A
B
C,D
E

Modification of the Amino Nitrogen. In Figure 3, the dose-response curves of the catecholamines protochylol, norepinephrine, epinephrine, isopropyl arterenol and N-methylepinephrine are shown. These agonists differ only in the substituents present on the nitrogen atom of the side chain. With the exception of N-methylepinephrine, the activity of the other compounds was enhanced by the substitution of alkyl groups as can be seen by comparing the dose-response curves of protochylol and isopropyl arterenol with the other agonists. Indeed, the large N-substituent as found in protochylol, the most potent agonist studied in this system, enhanced its affinity to a value approximately two-fold over isopropyl arterenol and was found to be 13 times that of epinephrine and norepinephrine. Methylation of norepinephrine (epinephrine) did not produce a significant alteration in the dose-response curves, however, methylation of epinephrine to form the tertiary amine, N-methyl epinephrine, produced a marked decrease in affinity. This single modification resulted in a greater than 1600-fold decrease in the molecule's ability to mobilize FFA. It is apparent that the ability of catecholamines to mobilize FFA is best associated with primary and secondary catecholamines, these findings being in agreement with the conclusions expressed by other investigators (94,95). To a lesser degree, the tertiary catecholamines are capable of eliciting a maximal response in this adipose tissue system.

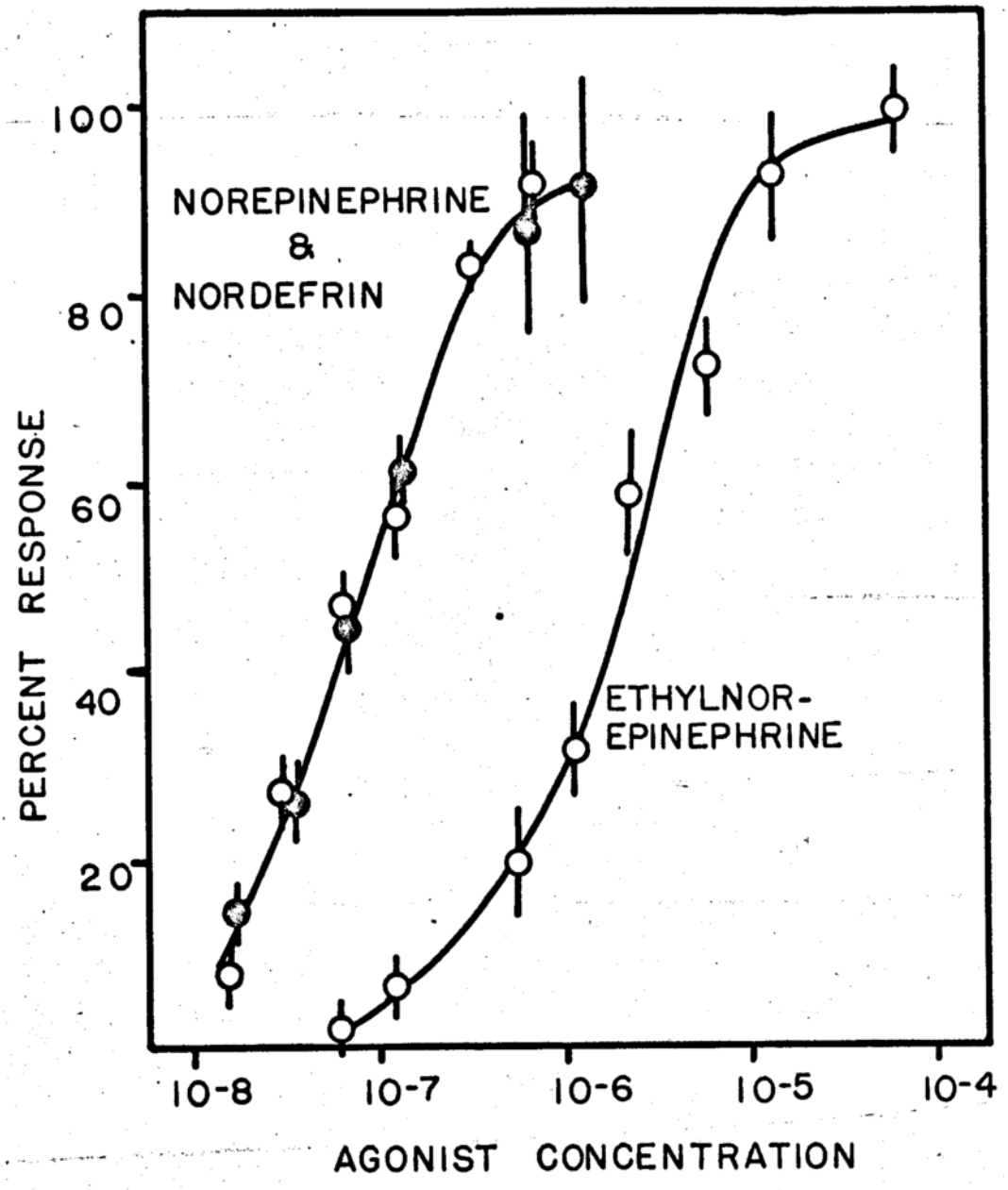
Modification of the Beta Carbon on the Ethylamino-Side Chain. Results obtained with the paired compounds norepinephrine and dopamine, epinephrine and adrenalone, octopamine and tyramine are illustrated in Figure 4. Norepinephrine and dopamine, and octopamine and tyramine,

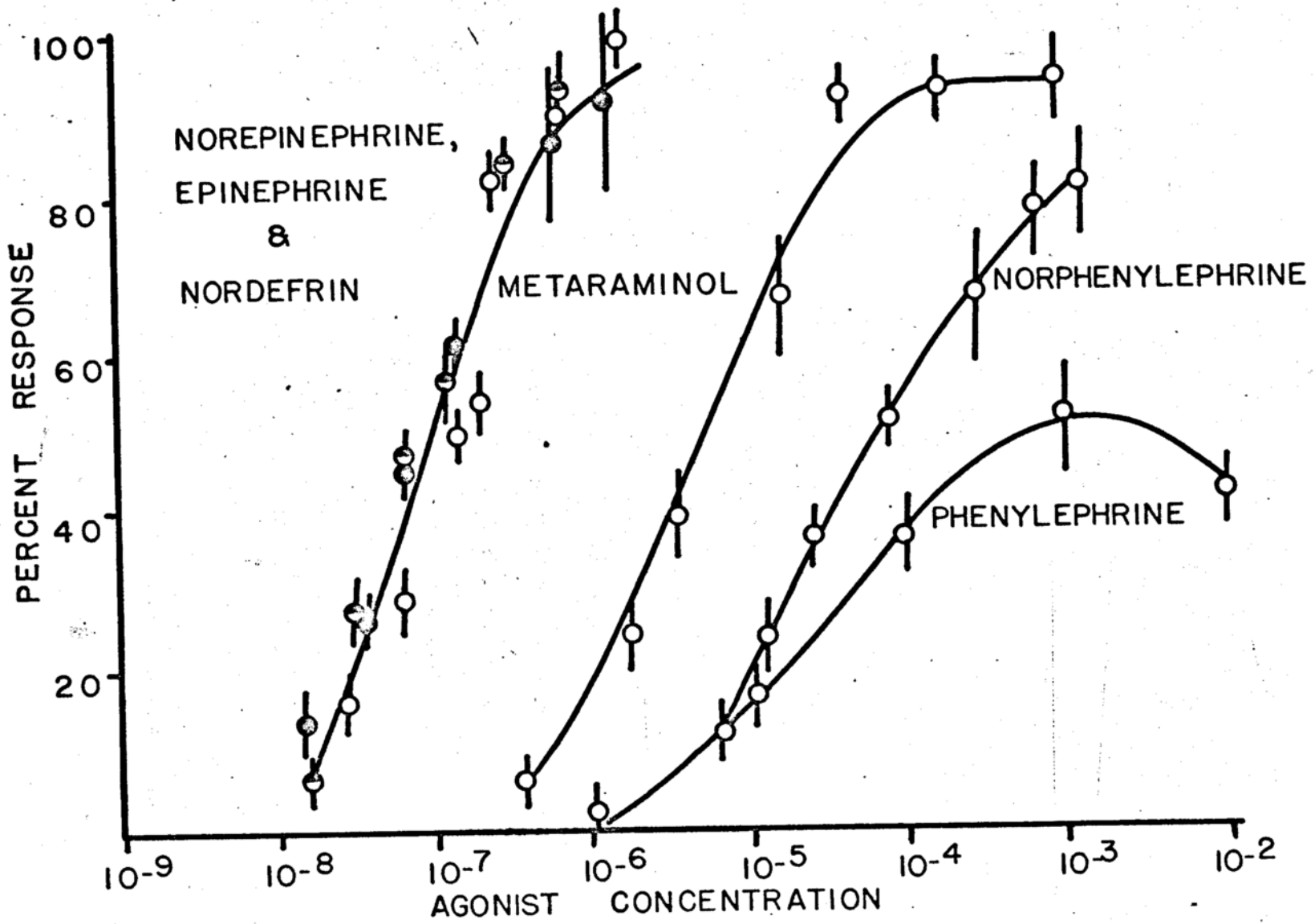

100
80
60
40
20
PERCENT RESPONSE
NOREPINEPHRINE
&
EPINEPHRINE
OCTOPAMINE
&
DOPAMINE
ADRENALONE
TYRAMINE
10-9
10-8
10-7
10-6
10-5
10-4
10-3
10-2
AGONIST CONCENTRATION

differ in the presence or absence of a hydroxyl function on the beta carbon. The removal of this chemical group from norepinephrine resulted in a 250-fold decrease in affinity as shown by the dose-response curve of dopamine, as compared to that of norepinephrine, whereas the removal of the hydroxyl function of octopamine completely abolished the ability of the molecule to mobilize FFA as indicated by the results obtained with tyramine. Adrenalone differs from epinephrine only in the conversion of the beta-hydroxyl group to a ketone function, yet these two compounds show a marked difference in their ability to release FFA. Adrenalone, possessing the ketone function, is about 400 times less effective than epinephrine. The presence of the side chain hydroxyl thus appears to enhance the mobilizing properties of the phenethylamine molecule and must play an important role in the binding of phenethylamine agonists to receptor material in adipose tissue.

Modification of the Alpha Carbon of the Ethylamine-Side Chain. In Figure 5, ethylation at this position, as can be seen with ethylnorepinephrine, resulted in a significant shift of the dose-response curve to the right, whereas methylation of the alpha carbon (nordefrin) did not influence the FFA mobilizing effect as is shown by comparison with the dose-response curve of norepinephrine. Ethylnorepinephrine was found to be approximately 20 times less effective than the agonists nordefrin and norepinephrine. The plotted results with the latter agonists are indistinguishable in this system, and therefore are regarded as being equipotent.

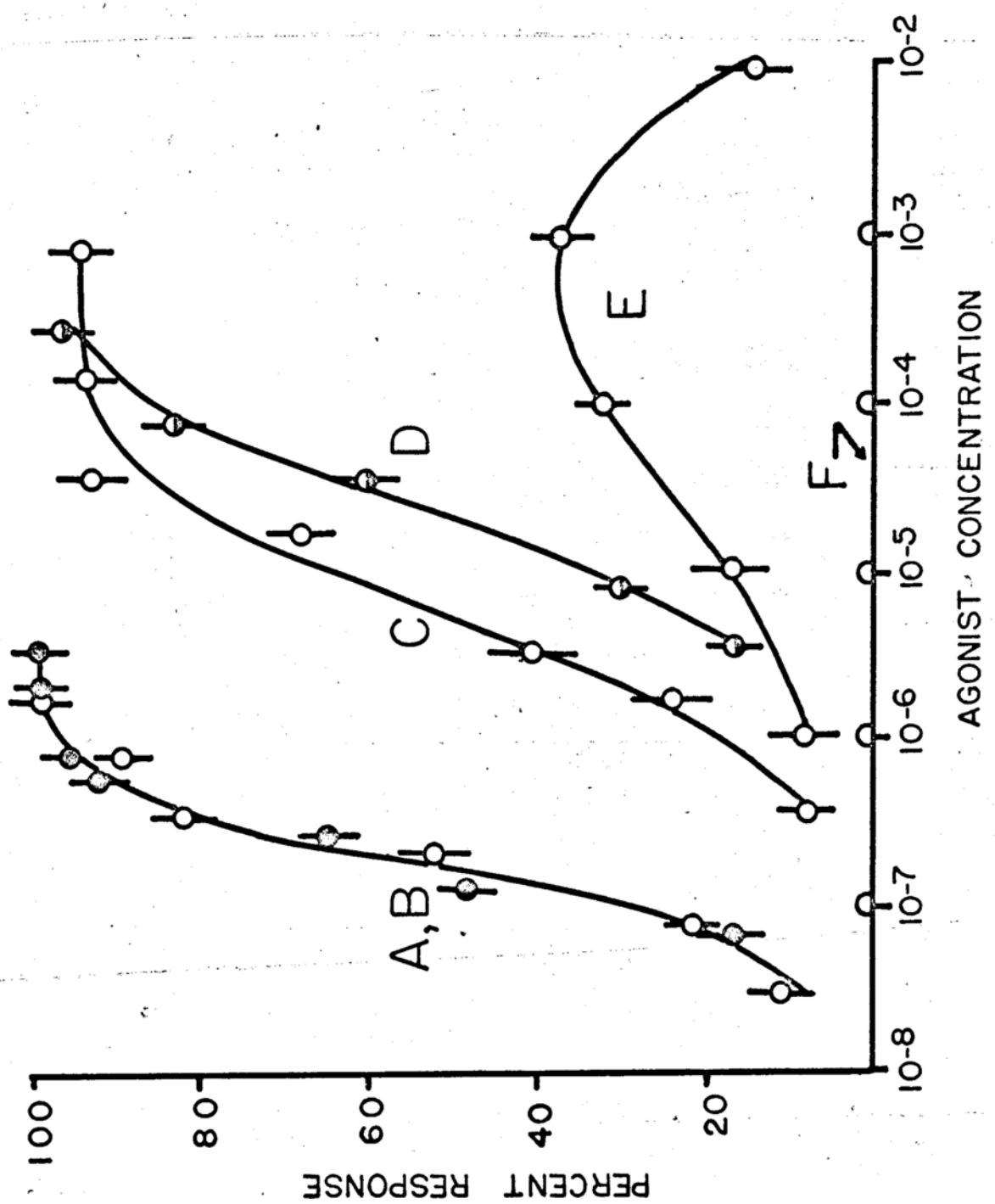
Modification of the Para- or 4-Position of the Phenyl Nucleus. As shown in Figure 6, the results obtained with the catecholamines


100
80
60
40
20
PERCENT RESPONSE
NOREPINEPHRINE
&
NORDEFRIN
ETHYLNOR-
EPINEPHRINE
10-8
10-7
10-6
10-5
10-4
AGONIST CONCENTRATION


100
80
60
40
20
PERCENT RESPONSE
NOREPINEPHRINE,
EPINEPHRINE
&
NORDEFRIN
METARAMINOL
NORPHENYLEPHRINE
PHENYLEPHRINE
10-9
10-8
10-7
10-6
10-5
10-4
10-3
10-2
AGONIST CONCENTRATION

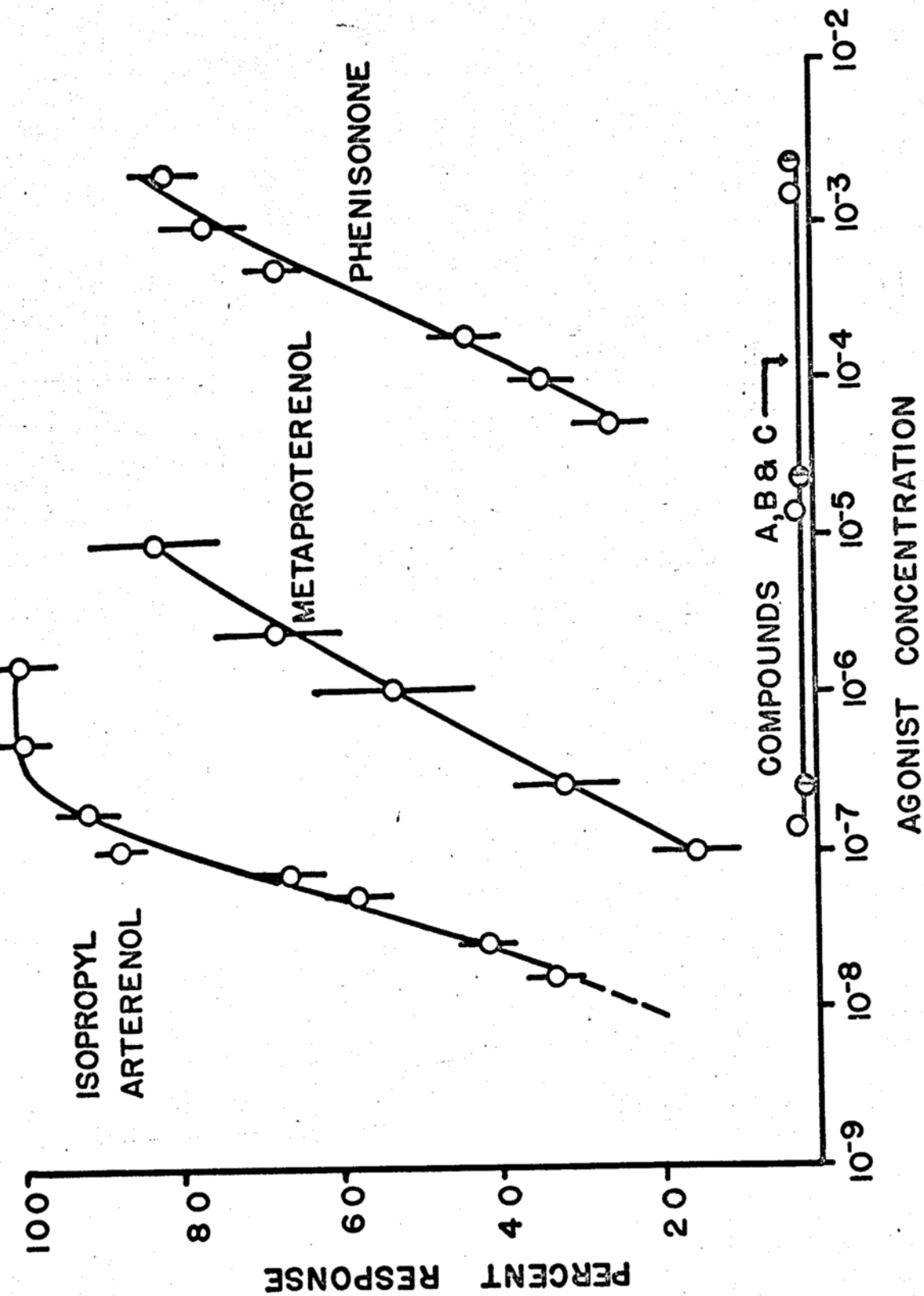
norepinephrine, epinephrine and nordefrin were not found to be significantly different in the mobilization of FFA from adipose tissue slices. On the other hand, the non-catecholamines norephenylephrine, phenylephrine and metaraminol, differing by the absence of phenolic group at the 4-position, show marked changes in their dose-response curves. The absence of this phenolic moiety produced a parallel shift in the curves to the right indicating a decrease in the affinity for the adipose tissue system, in vitro. Metaraminol was about 80-fold less effective and norphenylephrine was approximately 800-fold less active than nordefrin and norepinephrine, their corresponding hydroxylated analogues. Phenylephrine, in addition to a marked decrease in affinity as compared to epinephrine, was unable to maximally stimulate the release of FFA in this system. It is evident that removal of the para- or 4-hydroxyl function from the phenyl nucleus decreases the ability of the molecule to mobilise FFA.

The compounds phenylephrine and phenylpropanolamine (shown in Figure 7) possess properties which sharply differentiate them from the other agonists studied. These compounds exhibit the phenomena of auto-inhibition which manifests itself in a decreased FFA release as the concentration of agonists is increased beyond a certain value. Basu (124) and Wenke et al. (91) have reported that catecholamines, in very high doses, also exhibit auto-inhibition in adipose tissue, in vitro. Auto-inhibition has been explained by the further existence of interdependent receptors which interact in the presence of higher concentrations of agonist and antagonise the maximal response in the system (125). No attempt was made to examine the nature of this property in these compounds in this study.


PERCENT RESPONSE
100
80
60
40
20
10-8
10-7
10-6
10-5
10-4
10-3
10-2
AGONIST CONCENTRATION
A,B
C
D
E
F2

Modification of the Meta- or 3-Position of the Phenyl Nucleus. In Figure 7, the results obtained with the paired compounds norepinephrine and octopamine, dopamine and tyramine, metaraminol and phenylpropanolamine, and epinephrine and metanephrine are presented. The removal of the ~~Meta~~phenolic group from the phenyl nucleus of norepinephrine resulted in a 125-fold decrease in effectiveness as is illustrated by the results found with octopamine. The importance of this same modification is also evident from the results obtained with tyramine and phenylpropanolamine. Tyramine, lacking the meta-hydroxyl group, is unable to mobilize FFA to a measureable level in this system whereas dopamine is able to mobilize FFA maximally. The loss of this group from metaraminol, as in phenylpropanolamine, markedly altered the dose-response relationship. In addition, methylation of the meta-hydroxyl group of epinephrine as in metanephrine completely abolished FFA mobilizing activity.

Modification of Isopropyl Arterenol. Marked differences in affinities are noted in the dose-response curves obtained for the agonists isopropyl arterenol, metaproterenol and phenisonone as is shown in Figure 8. A 30-fold difference in effective concentrations of the compounds metaproterenol and isopropyl arterenol was obtained, yet these compounds differ only in that metaproterenol has the two hydroxyl groups in the meta-positions of the phenyl nucleus. Phenisonone, an analogue of isopropyl arterenol which possesses a beta ketone and an alpha methyl group on the side chain, is about 10,000 times less effective. These data substantiate the importance of the para-phenolic group and the presence of beta-hydroxylation on the

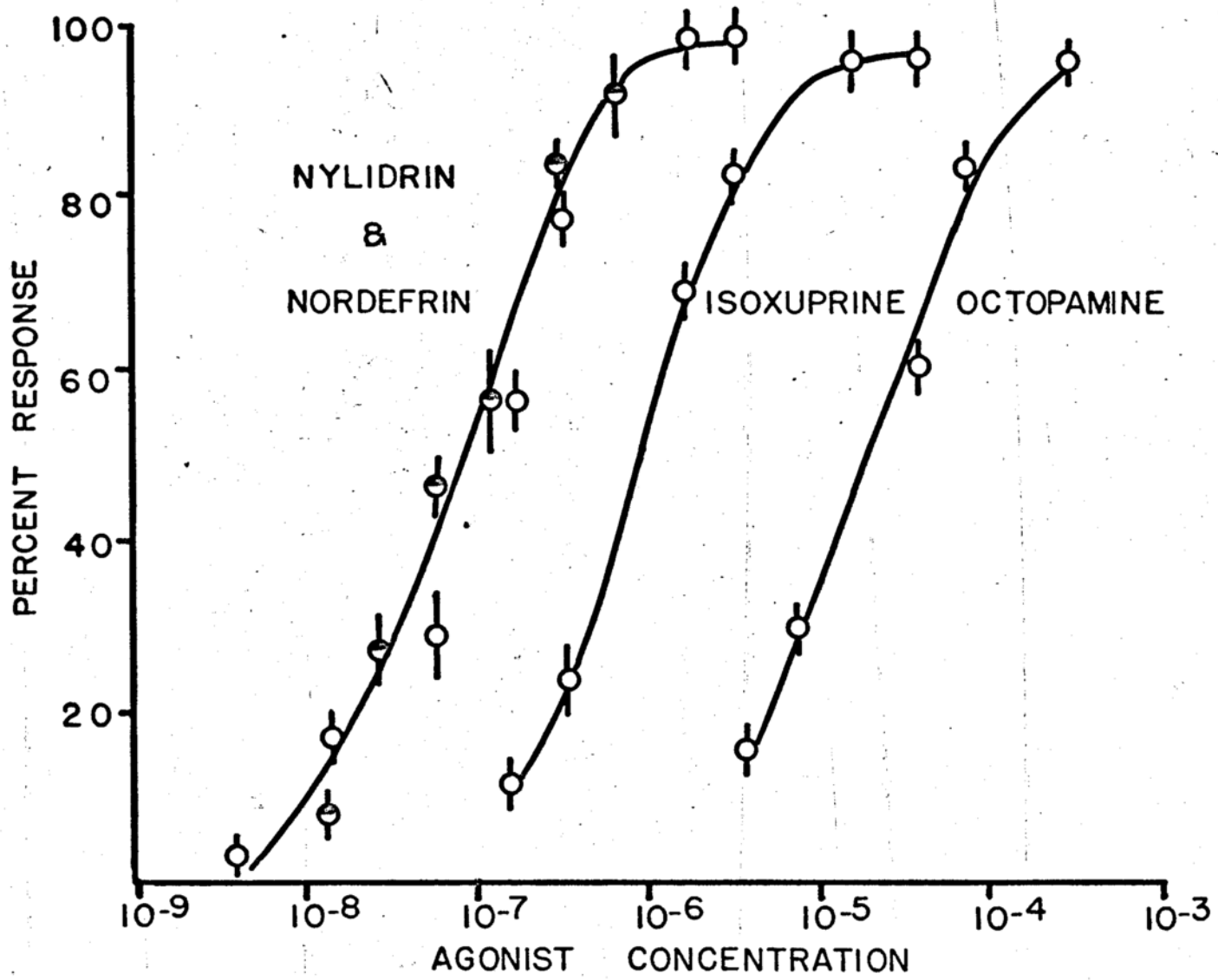

PERCENT RESPONSE
100
80
60
40
20
ISOPROPYL ARTERENOL
METAPROTERENOL
PHENISONONE
COMPOUNDS A, B & C
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
10⁻³
10⁻²
AGONIST CONCENTRATION

ethylamino-side chain in the interaction of agonist and receptor in the adipose tissue system.

Removal of the phenyl nucleus of isopropyl arterenol as in 2-isopropylaminoethanol completely abolished FFA mobilizing activity. Increasing the size of the substituent on the amino nitrogen of ethanolamine failed to elevate FFA to a measureable level as indicated by the results obtained with N-benzylethanolamine and N-α-benzyl-monoethanolamine.

Modification of the Amino Nitrogen of Non-Catecholamines. In Figure 9, the results obtained for the compounds nordefrin, nylidrin, isoxuprine and octopamine are shown. A marked increase in FFA mobilizing activity is noted with the non-catecholamines nylidrin and isoxuprine as compared with the dose-response curve of octopamine, another non-catecholamine which lacks a substituent on the nitrogen and alpha carbon atoms of the ethylamino-side chain. The activity of these agonists appears to be associated with the presence of a large aralkyl substituent on the nitrogen atom. Nylidrin is equipotent with the catecholamines norepinephrine, epinephrine and nordefrin whereas isoxuprine is approximately one-fifth as active. Nordefrin, possessing the catechol moiety, but lacking an N-substituent, was included for comparative purposes with isoxuprine and nylidrin.

The present study has shown that all catecholamines were capable of inducing a maximal FFA releasing response in the adipose tissue system, in vitro. Modification of the basic catecholamine structure produced marked changes in affinity and to a much lesser extent, in intrinsic activity. It is apparent that any model system designed to illustrate the interaction of various substituted phenethylamines, and


100
80
60
40
20
PERCENT RESPONSE
NYLIDRIN
&
NORDEFRIN
ISOXUPRINE
OCTOPAMINE
10-9
10-8
10-7
10-6
10-5
10-4
10-3
AGONIST CONCENTRATION

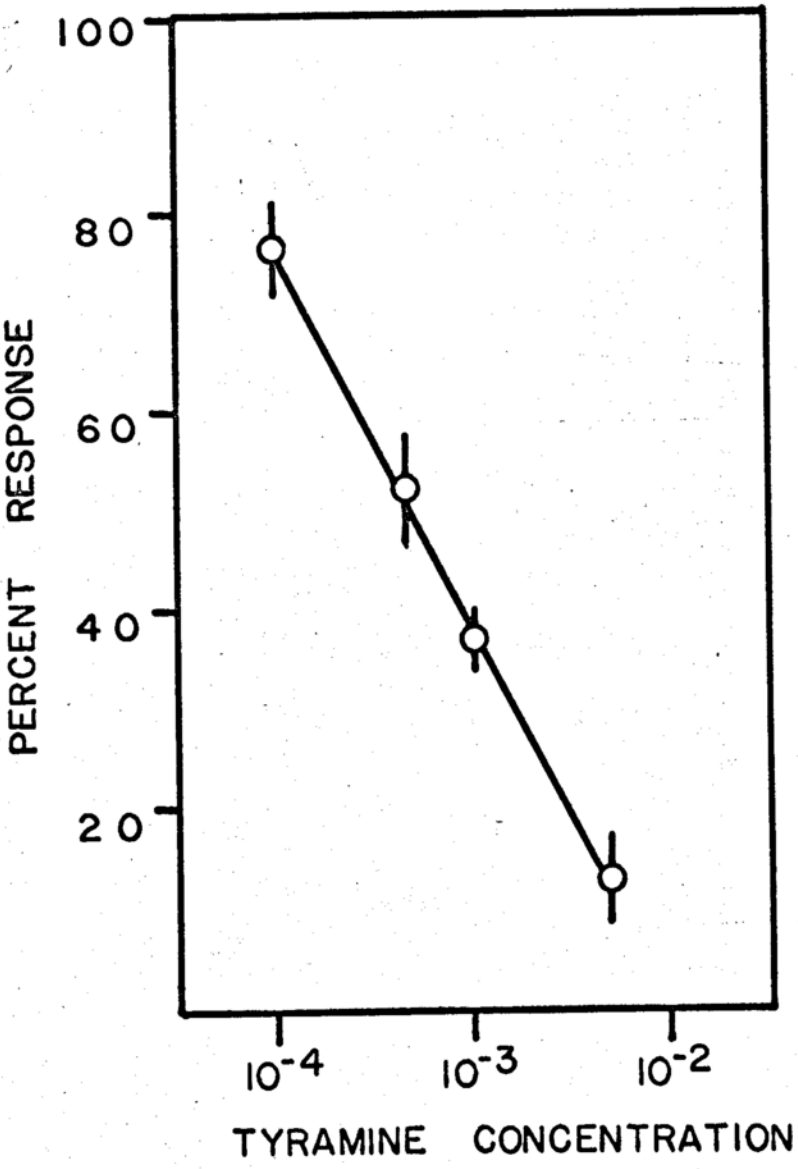
in particular the catecholamines, with the adrenergic-adipose tissue receptor system must take into account the presence or absence of chemical groups on the nitrogen atom, the alpha and beta carbon atoms of the side chain, and the meta- and para-positions of the phenyl nucleus. The observed decreases in FFA mobilizing activities resulting from the removal of a phenolic group or beta-hydroxyl group from the catecholamine molecule are in agreement with the studies by Mueller and Horwitz (95) in human subjects and Rudman et al. (94) in hamster adipose tissue, in vitro.

Effect of Tyramine on Catecholamine-Induced Lipolysis from Adipose Tissue Slices. Several compounds including tyramine, amphetamine and ephedrine were found to be incapable of elevating FFA to a measureable level in this system (See Table I). The negative results obtained with tyramine confirm earlier reports by Love et al. (100) and others (50, 126). In addition to their structural similarities to the more potent phenethylamines, these compounds were chosen because one of their proposed mechanisms of action is an indirect action mediated by the release of endogenous catecholamines (127). Since Paoletti et al. (47) and others (48,49) have shown that adipose tissue contains appreciable quantities of endogenous norepinephrine, it was expected that some activity would be observed with the indirect acting compounds at the high concentrations employed in this study.

The absence of an observable response with these compounds may be interpreted in at least two ways: (1) the inability of these compounds to stimulate the release of FFA in vitro may be due to a failure of these compounds to reach the endogenous stores of norepinephrine or

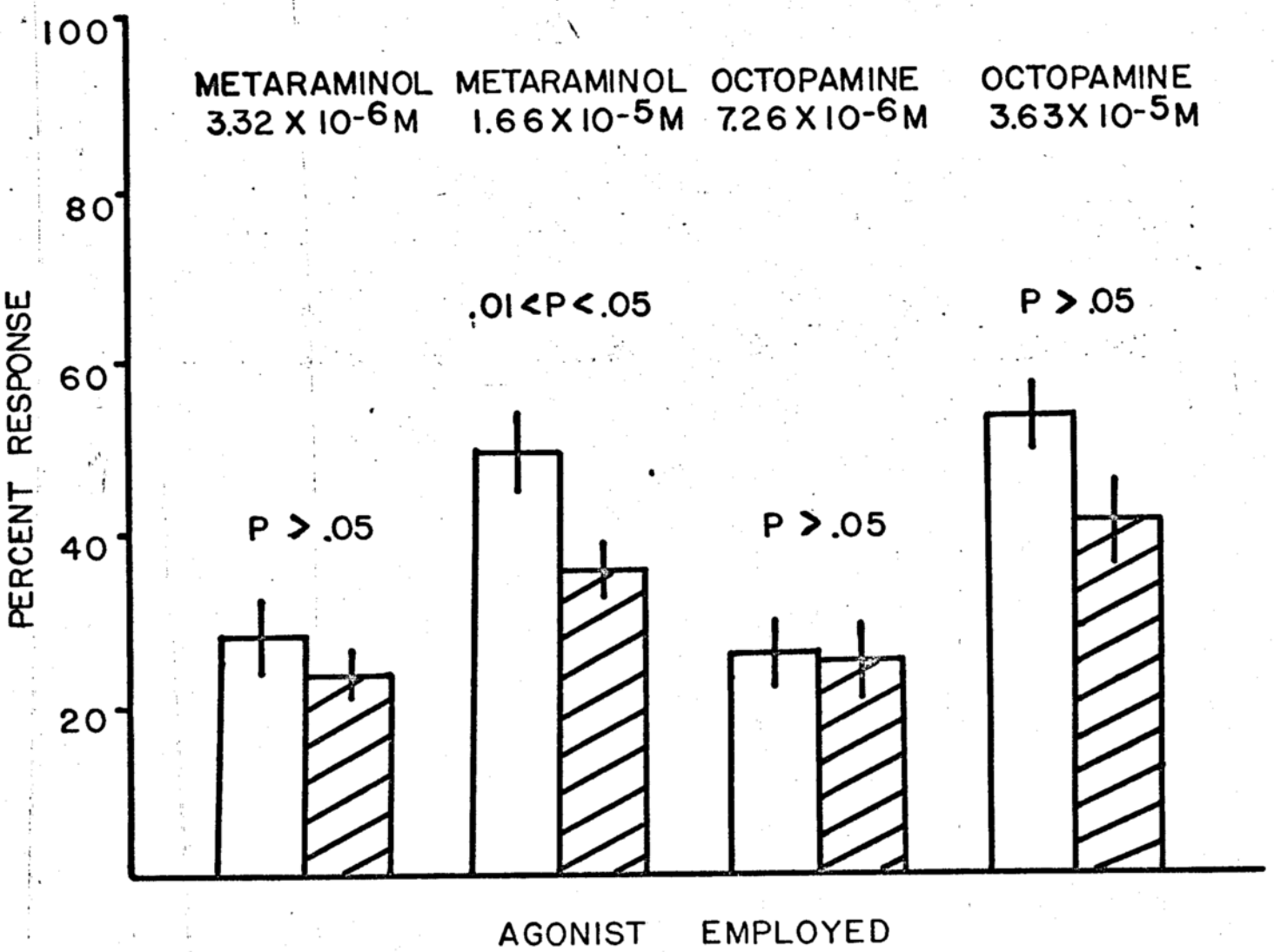
(2) if they reached norepinephrine stores, they failed to cause the release of sufficient amounts of endogenous catecholamine. The former possibility suggests that the compounds failed to diffuse through adipose tissue or that they were accumulated preferentially by the tissue, thus being effectively removed from the aqueous phase within the cells. The latter alternative, involving the failure of these compounds to cause release of endogenous catecholamines is in direct opposition to the currently offered explanation of the mechanism of action of these compounds. In an attempt to explain the lack of a measureable response, tyramine was examined for its effect on norepinephrine-induced lipolysis.

Various concentrations of tyramine were preincubated prior to the addition of a high concentration of norepinephrine (5.2×10^{-7} M) and the FFA release rate determined. It is apparent from the data shown in Figure 10 that tyramine effectively antagonized norepinephrine-induced lipolysis and that the release of FFA was inhibited by approximately 50% in the presence of 5×10^{-4} M tyramine. The observed antagonism precludes the possibility that tyramine failed to diffuse through the fat tissue. However, these data do suggest that the tyramine was indeed incapable of releasing the endogenous catecholamines found in the adipose tissue and further imply that the adipose tissue system differs to some extent from the adrenergic systems of heart, brain, etc. where tyramine has been shown to release endogenous catecholamines. Thus, it has been shown that tyramine interacts with the adrenergic receptor system in adipose tissue and can best be described as an antagonist rather than an agonist.


100
80
60
40
20
PERCENT RESPONSE
10-4
10-3
10-2
TYRAMINE CONCENTRATION

Effect of Reserpine Pretreatment on Metaraminol and Octopamine Mobilization, in vitro. In addition to tyramine, ephedrine and amphetamine, the agonists nordefrin, octopamine and metaraminol also have been reported to have an indirect component of action. Although the storage sites for norepinephrine in sympathetic neurons are relatively specific, these agonists possess the structural requirements necessary to displace norepinephrine and occupy the storage sites of norepinephrine (128). Recent evidence suggests that this indirect action resides more specifically in the L(+) isomers of metaraminol and nordefrin, whereas the direct component of action is associated with the D(-) isomers (129). It was of interest to determine the FFA mobilizing effects of these agents in adipose tissue depleted of norepinephrine content by pretreatment with reserpine. Reserpine has been shown to deplete catecholamines from adipose tissue as well as other tissues (47,48,49).

Animals were pretreated with reserpine (5 mg./kg. i.p.) and fasted for 16-20 hours prior to the experiment. Animals not given reserpine were treated similarly and served as controls. The pretreated animals exhibited marked visual signs of ptosis, sedation, salivation and defecation, all characteristic of the syndrome produced by reserpine and associated with depletion of biogenic amines. Two concentrations of each agonist were employed in the in vitro phase of this experiment as can be seen in Figure 11. Although the results with metaraminol at a concentration of 1.66×10^{-5} M, differed significantly from those obtained with the controls, the data support the concept that these compounds act primarily by a direct mechanism and not by the release of endogenous norepinephrine. Thus, the


100
80
60
40
20
PERCENT RESPONSE
METARAMINOL 3.32 X 10-6M
METARAMINOL 1.66 X 10-5M
OCTOPAMINE 7.26 X 10-6M
OCTOPAMINE 3.63 X 10-5M
P > .05
.01 < P < .05
P > .05
P > .05
AGONIST EMPLOYED

response obtained in the reserpine treated tissue was approximately the same as that obtained in the controls.

The above experiments conducted with the indirect acting sympathomimetics suggest that the mobilization of FFA from adipose tissue may represent an adrenergic system unique in the storage of catecholamines. The indirect-acting sympathomimetics employed in this study were either inactive in mobilizing FFA (tyramine, amphetamine, ephedrine) or did not have their activity decreased by chemical sympathectomy (metaraminol and octopamine). This should not be construed to imply that the physiological amounts of catecholamines in adipose tissue are negligible in their functional aspects, since an abundance of evidence points to the importance of the sympathetic nervous system in the mobilization of FFA (45,46,47,48,50,51,53,54,55).

Double Reciprocal Relationships. The data illustrated in the dose-response relationships (Figures 3-9) can be presented graphically in a form identical to the Lineweaver-Burk double reciprocal plot so common to enzyme systems (130). Taking the reciprocal of each side of the equation presented previously (See page 30) yields:

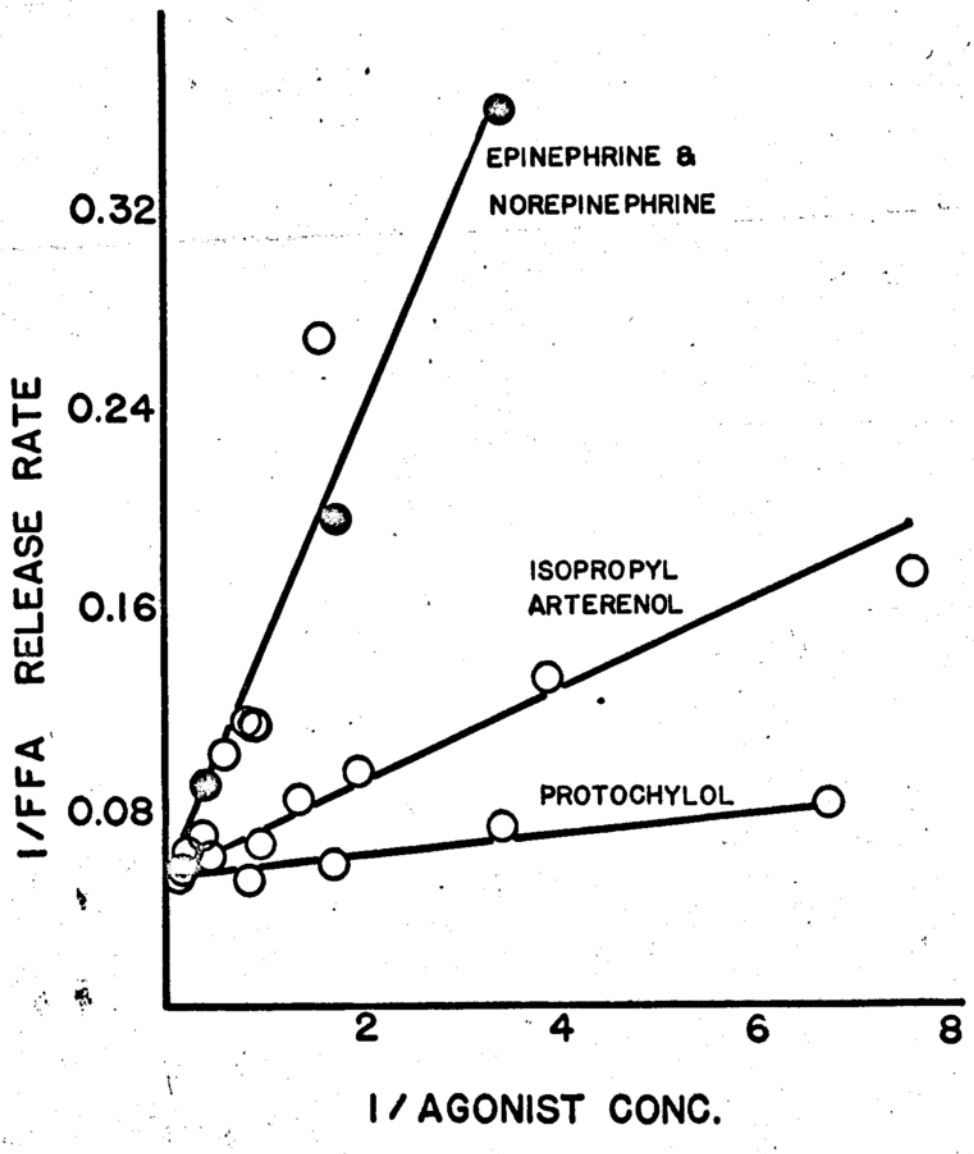
$$\frac{1}{R} = \frac{Ka}{I}\ \frac{1}{A} + \frac{1}{I}$$

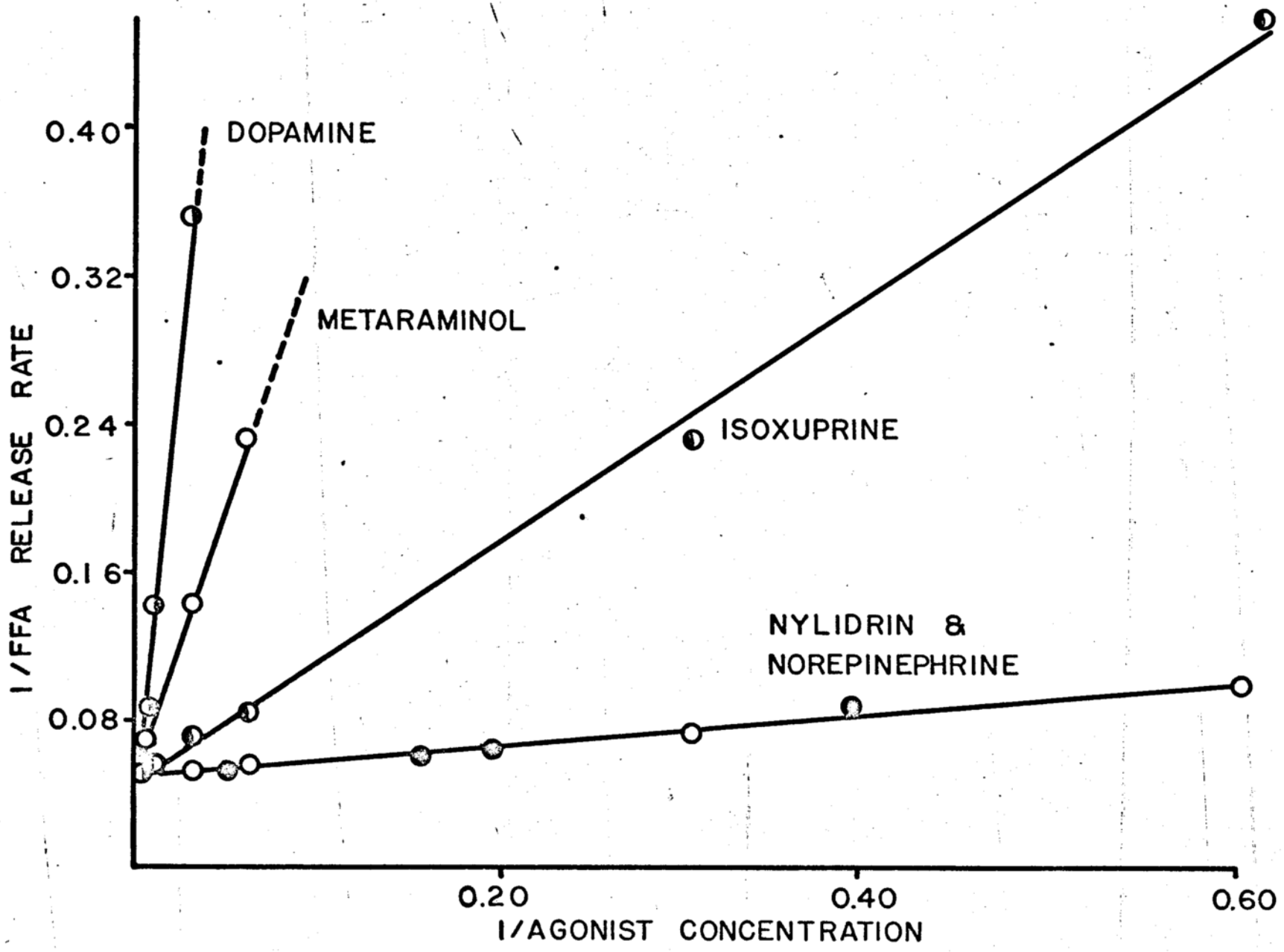
Plotting the data as 1/R versus 1/A yields a linear function with a slope numerically equal to Ka/I and an intercept of 1/I. Analysis of the data in this manner provides an efficient means of calculating the two parameters of agonist-receptor interactions, the intrinsic activity (I) and the affinity constant (1/Ka) from the intercept and the slope of the line, respectively. With the exception of phenylephrine and

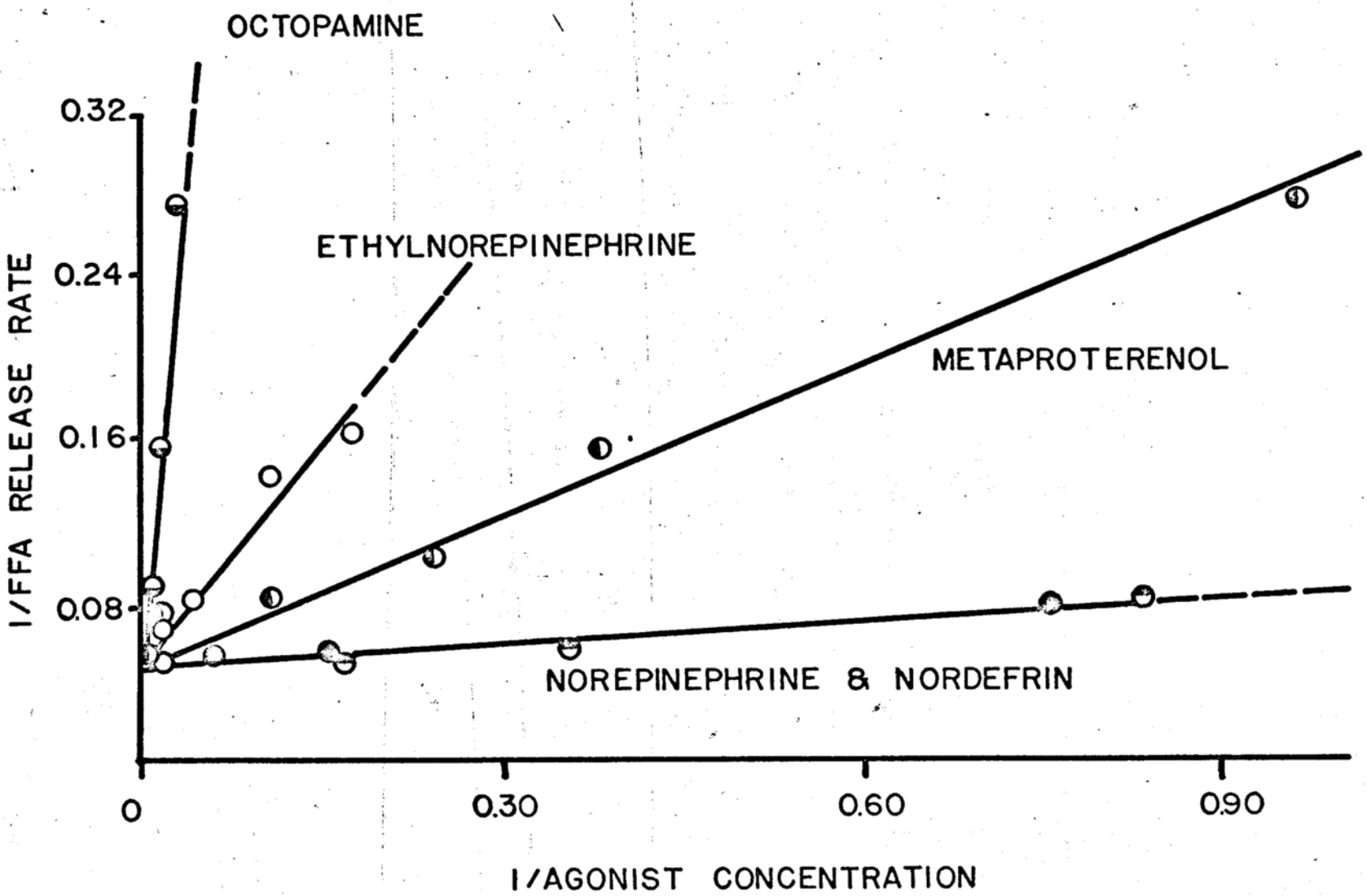
phenylpropanolamine, the data obtained with the agonists in these studies were plotted in this manner and are shown in Figures 12-15.

The data for norepinephrine are included in Figures 12-14 for comparative purposes, since a different scale for the abscissa was employed to permit plotting of other agonists. It is apparent from these graphs that (a) the lines representing the agonists all extrapolate to an identical ordinate intercept, indicating the same intrinsic activity for all compounds and (b) that the majority of agonists differ in the slopes of their lines, indicating differences in affinity for the adipose tissue system. The plotting of the data in this manner serves as check on the general agreement of the data with the equation expressed on page 30 and facilitates the calculation of the intrinsic activity and affinity constants.

Intrinsic Activity and Affinity Constants. The structure-activity relationships obtained in this study are summarized in Table I. Such a tabulation of the data permits a quantitative and visual comparison of the effects of the various agonists employed. The intrinsic activity constants have been expressed as the ratio of the maximal response obtainable with the particular agonist to the maximal response obtainable in this system (123), and the affinity constants (the reciprocal of the apparent drug-receptor dissociation constant, 1/Ka) are expressed as the pD_2 values of Miller, Becker and Tainter (131) defined as the negative logarithm of the agonist concentration required to produce a response equal to 50% of its maximal response in this system. These constants can be derived either from the dose-response relationships or directly from the slope and intercept value of the lines from the double reciprocal plots. As shown in the table,


EPINEPHRINE &
NOREPINEPHRINE
ISOPROPYL
ARTERENOL
PROTOCHYLOL
0.32
0.24
0.16
0.08
1/FFA RELEASE RATE
2
4
6
8
1/AGONIST CONC.


0.40
0.32
0.24
0.16
0.08
1/FFA RELEASE RATE
DOPAMINE
METARAMINOL
ISOXUPRINE
NYLIDRIN &
NOREPINEPHRINE
0.20
0.40
0.60
1/AGONIST CONCENTRATION


OCTOPAMINE
ETHYLNOREPINEPHRINE
METAPROTERENOL
NOREPINEPHRINE & NORDEFRIN
0.32
0.24
0.16
0.08
1/FFA RELEASE RATE
0
0.30
0.60
0.90
1/AGONIST CONCENTRATION

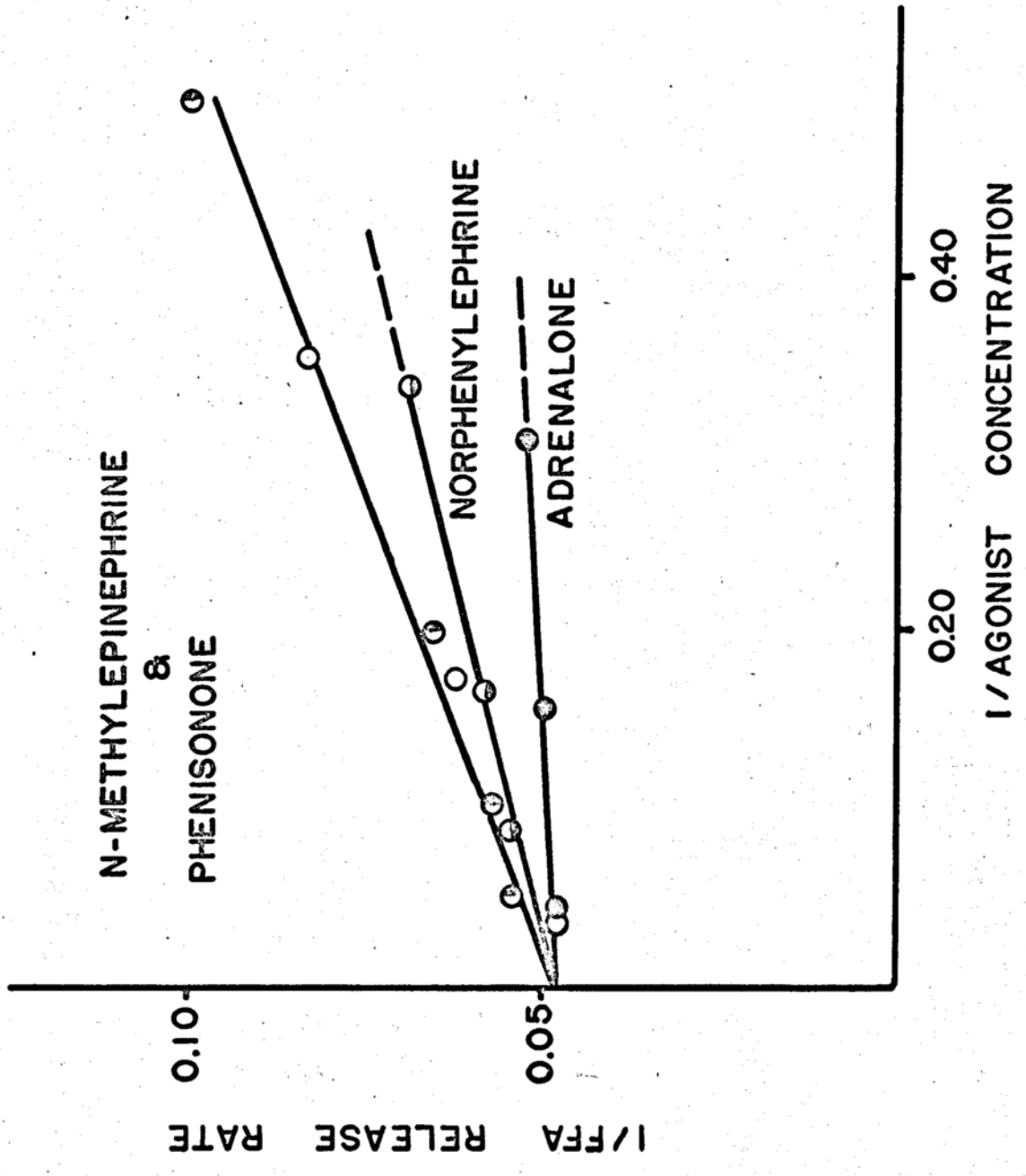
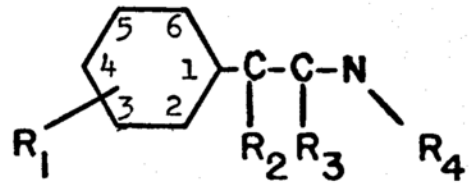

N-METHYLEPINEPHRINE
&
PHENISONONE
NORPHENYLEPHRINE
ADRENALONE
0.10
0.05
1/FFA RELEASE RATE
0.20
0.40
1/AGONIST CONCENTRATION

TABLE I.--Intrinsic Activity and Affinity (pD_2) Constants for Agonists on the Mobilization of Free Fatty Acids from Rat Adipose Tissue



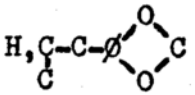
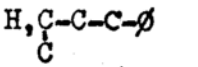
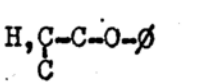
Compound	Name	R_1	R_2	R_3	R_4	I[a]	pD_2
1	Protochylol	3-OH,4-OH	OH	H		1.0	7.9
2	Isopropyl arterenol	3-OH,4-OH	OH	H	H,$CH(CH_3)_2$	1.0	7.6
3	Norepinephrine	3-OH,4-OH	OH	H	H,H	1.0	7.1
4	Epinephrine	3-OH,4-OH	OH	H	H,CH_3	1.0	7.1
5	Nordefrin	3-OH,4-OH	OH	CH_3	H,H	1.0	7.1
6	Nylidrin	4-OH	OH	CH_3		1.0	7.1
7	Isoxuprine	4-OH	OH	CH_3		1.0	6.1

TABLE I (continued)

8	Metaproterenol	3-OH,5-OH	OH	H	H,$CH(CH_3)_2$	1.0	6.1
9	Ethylnorepinephrine	3-OH,4-OH	OH	CH_2CH_3	H,H	1.0	5.8
10	Metaraminol	3-OH	OH	CH_3	H,H	1.0	5.2
11	Octopamine	4-OH	OH	H	H,H	1.0	4.7
12	Dopamine	3-OH,4-OH	H	H	H,H	1.0	4.4
13	Adrenalone	3-OH,4-OH	=O	H	H,CH_3	1.0	4.2
14	Norphenylephrine	3-OH	OH	H	H,H	1.0	4.2
15	Phenisonone	3-OH,4-OH	=O	CH_3	H,$CH(CH_3)_2$	1.0	3.6
16	N-methylepinephrine	3-OH,4-OH	OH	H	CH_3,CH_3	1.0	3.6
17	Phenylpropanolamine		OH	CH_3	H,H	0.37	4.7
18	Phenylephrine	3-OH	OH	H	H,CH_3	0.50	4.3
19	Amidephrine	3-$NHSO_2CH_3$	OH	H	H,CH_3	0.50	---
20	Phenethylamine		H	H	H,H	0.0	---

TABLE I (continued)

21	Metanephrine	3-OCH_3,4-OH	OH	H	H,CH_3	0.0	---
22	Tyramine	4-OH	H	H	H,H	0.0	---
23	Homarylamine	3,4-dioxymethylene	H	H	H,CH_3	0.0	---
24	Ephedrine		OH	CH_3	H,CH_3	0.0	---
25	Amphetamine		H	CH_3	H,H	0.0	---
26*	3,4-dihydroxymandelic acid					0.0	---
27*	3-methoxy,4-hydroxymandelic acid					0.0	---
28*	N-α-methylbenzylmonoethanolamine					0.0	---
29*	2-isopropylaminoethanol					0.0	---
30*	N-benzylethanolamine					0.0	---

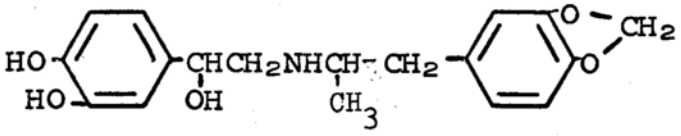
[a]Intrinsic activity constant. *Chemical structure is shown in Table II.

with the exception of amidephrine, phenylpropanolamine and phenylephrine, all active compounds were able to maximally stimulate FFA release and therefore possess an intrinsic activity constant of unity (1.0). Amidephrine, in the highest concentration employed, was able to mobilize approximately 50% of the maximum release of the system and thus is represented by an intrinsic activity constant of 0.50. On the other hand, structural modification produced marked changes in the pD_2 values, ranging from 3.6 for N-methylepinephrine and phenisonone to 7.9 for the most potent agonist, protochylol. All inactive compounds employed are included in the data and are represented by an intrinsic activity constant of zero (0.0). For clarification of structural modifications of the various phenethylamines, the chemical structures of all compounds are illustrated in Table II.

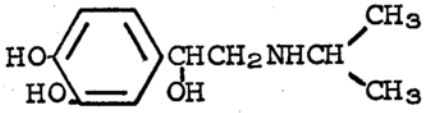
The data in Table I indicate the effect structural modification of the basic phenethylamine nucleus had on the ability of a compound to stimulate the mobilization of FFA from adipose tissue, in vitro. The parent compound, phenethylamine, was found to be completely inactive in this system. Alpha-methylation (amphetamine) or para-hydroxylation (tyramine) of phenethylamine did not increase activity to a measureable level, while beta-hydroxylation along with alpha-methylation had variable results (compare the slightly active phenylpropanolamine with the inactive ephedrine). Placing a meta-hydroxyl group on the phenyl ring of tyramine (dopamine) or phenylpropanolamine (metaraminol) significantly increased the FFA mobilizing activity. Para-hydroxylation of metaraminol (nordefrin) or the combination of para-hydroxylation and N-substitution of phenylpropanolamine (isoxuprine and nylidrin) markedly enhanced the lipolytic activity possessed by the agonist molecule.

TABLE II. Chemical Structures of Compounds Utilized in this Study

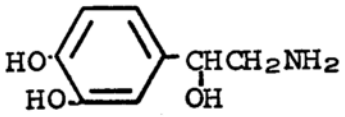
1. Protochylol



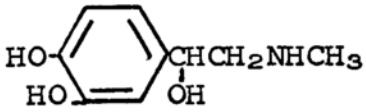
2. Isopropyl arterenol



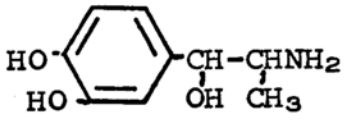
3. Norepinephrine



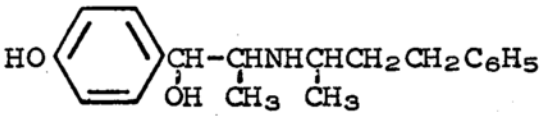
4. Epinephrine



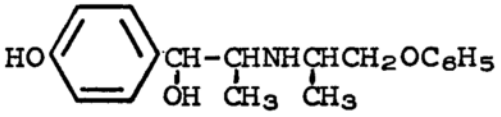
5. Nordefrin



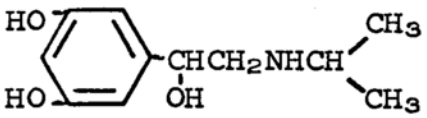
6. Nylidrin



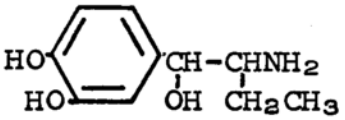
7. Isoxuprine



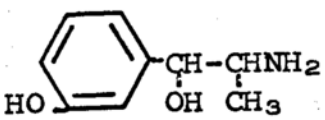
8. Metaproterenol



9. Ethylnorepinephrine



10. Metaraminol



11. Octopamine

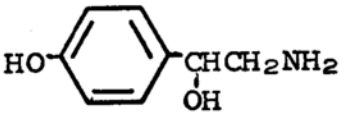
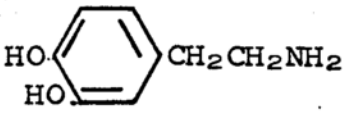
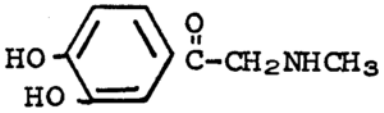


TABLE II (continued)

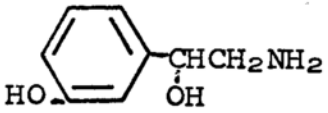
12. Dopamine



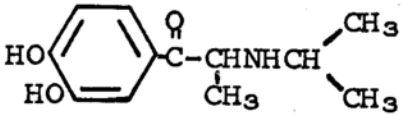
13. Adrenalone



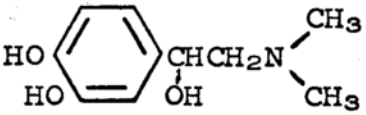
14. Norphenylephrine



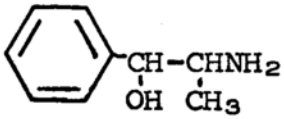
15. Phenisonone



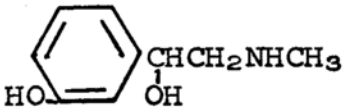
16. N-methylepinephrine



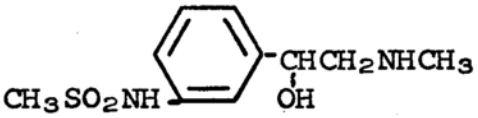
17. Phenylpropanolamine



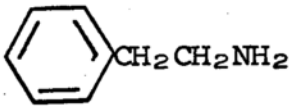
18. Phenylephrine



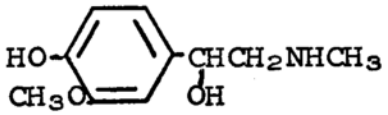
19. Amidephrine



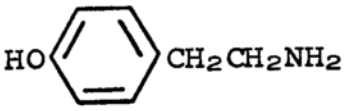
20. Phenethylamine



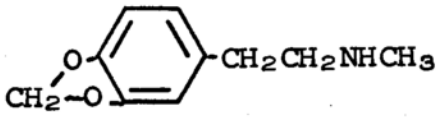
21. Metanephrine



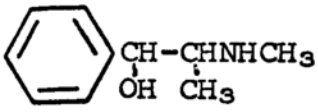
22. Tyramine



23. Homarylamine



24. Ephedrine



25. Amphetamine

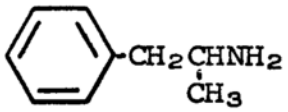
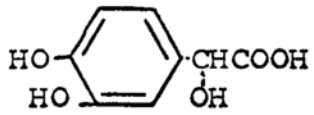
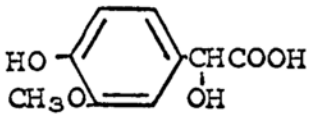


TABLE II (continued)

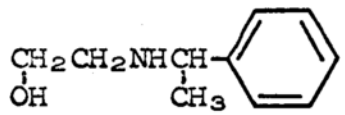
26. 3,4-dihydroxymandelic acid



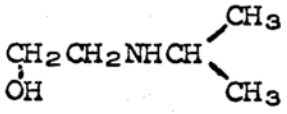
27. 3-methoxy,4-hydroxymandelic acid



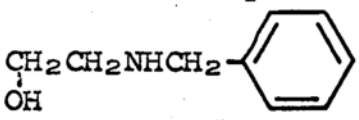
28. N- α-methylbenzylmonoethanolamine



29. 2-isopropylaminoethanol



30. N-benzylethanolamine



It is evident that beta-hydroxylation coupled with the presence of the catechol nucleus conveys optimal activity upon the phenethylamine molecule as can be seen by the large pD_2 values possessed by these type compounds (e.g. epinephrine, norepinephrine, and nordefrin). The affinity of the basic catecholamine molecule (norepinephrine) was enhanced by the presence of large or bulky alkyl or aralkyl functions upon the nitrogen atom of the side chain (protochylol and isopropyl arterenol).

A significant decrease in the pD_2 values by structural modification of the catecholamines (norepinephrine, epinephrine, isopropyl arterenol, and nordefrin) was noted: (1) by the presence of a tertiary amine group (N-methylepinephrine), (2) by the absence of either one of the ring hydroxyl functions (metaraminol, octopamine, and norphenylephrine), (3) by the absence of the beta-hydroxyl group (dopamine) or by the presence of a ketone function (phenisonone and adrenalone) on the ethylamino-side chain, (4) by the substitution of an ethyl group on the alpha carbon of the ethylamino-side chain (ethylnorepinephrine), (5) by an alteration in the positions of the two-hydroxyl functions on the phenyl ring (metaproterenol), and (6) by the absence of the phenyl nucleus (N-a-methylbenzylmonoethanolamine, N-benzylethanolamine and 2-isopropylaminoethanol).

It may also be noted in Table I that methylation of the meta-hydroxyl group (metanephrine) and/or the removal of the nitrogen atom (3,4-dihydroxymandelic acid and 3-methoxy,4-hydroxymandelic acid) of epinephrine completely abolished FFA mobilizing activity and that phenethylamine molecules lacking a free phenolic function (amidephrine, homarylamine, amphetamine, phenylpropanolamine and ephedrine) possess

little or no FFA mobilizing activity. These data support the proposal that the catechol nucleus and nitrogen atom are necessary chemical groups for optimal activity of the phenethylamine molecule in this adipose tissue system.

Analysis of these data reveal that an enhancement of affinity (an increase in pD_2 values) was noted by the following structural modifications of the phenethylamine molecule: (a) an increase in the size of alkyl or aralkyl monosubstituents on the nitrogen atom of the ethylamino-side chain, (b) the presence of an alcoholic or beta carbon hydroxyl function on the ethylamino-side chain, whereas its absence or the presence of a ketone function is incompatible with potent FFA releasing activity, (c) the presence of a meta- or 3-hydroxyl group on the phenyl nucleus, (d) the presence of a para- or 4-hydroxylation on the phenyl nucleus, and (e) in the absence of an alkyl substituent larger than a methyl group on the alpha carbon of the side chain. A partial or complete loss of FFA mobilizing activity as indicated by decreased affinity and/or intrinsic activity constants was observed in the following structural modifications: (a) in the absence of the catechol group on the phenethylamine molecule, (b) in the absence of the phenyl nucleus, (c) in the absence of the nitrogen atom on the ethylamino-side chain, and (d) by the presence of a tertiary amine group on the amino nitrogen of the side chain.

Summary. The increasing importance of the mobilization of FFA as a contributing factor in certain disease states and as a primary energy source in body metabolism has led to considerable emphasis being placed on the role of adipose tissue in FFA transport. The sympa-

thetic nervous system and in particular, the adrenergic neurohormones play a significant role in the control of lipid metabolism. Recent work has shown that catecholamine-induced lipolysis occurs along pathways similar to the processes elucidated by Sutherland and Rall (67, 68), that is, a stimulation of the conversion of adenosine triphosphate (ATP) to 3',5'-cyclic-AMP, which in turn, catalyzes the transformation of an inactive lipase to an active lipase in adipose tissue (40,56,64). The activated lipase enzyme further catalyzes the hydrolysis of triglycerides to FFA intracellularly. A better understanding of the chemical specificity of the substituted phenethylamine molecule was sought for future development of therapeutic agents modifying FFA mobilization.

The log dose-response and double reciprocal relationships for agonists reported herein conform to the theory of interaction involving receptor and agonist in a reversible complex (121,123). The calculations of the intrinsic activity and affinity constants for the adrenergic-adipose tissue receptor system, in vitro, are useful parameters of the agonist-receptor interaction to determine the structural features pertinent to FFA mobilizing activity.

The structure-activity relationships can be discussed with reference to alpha- and beta-type adrenergic receptors and it is apparent that adipose tissue responds more favorably to the beta-type adrenergic stimulants. Of the alpha agonists, norepinephrine, phenylephrine and amidephrine, the latter two possess only weak mobilizing activity, whereas norepinephrine is a potent agonist. On the other hand, a large number of beta-stimulants are effective in this system and include isopropyl arterenol, nylidrin, isoxuprine, metaproterenol

and protochylol. As reported (90,92) and confirmed by Cernohorsky et al. (93), the increase in affinity obtained by the addition of bulky groups on the nitrogen atom is characteristic of a beta-type adrenergic receptor system.

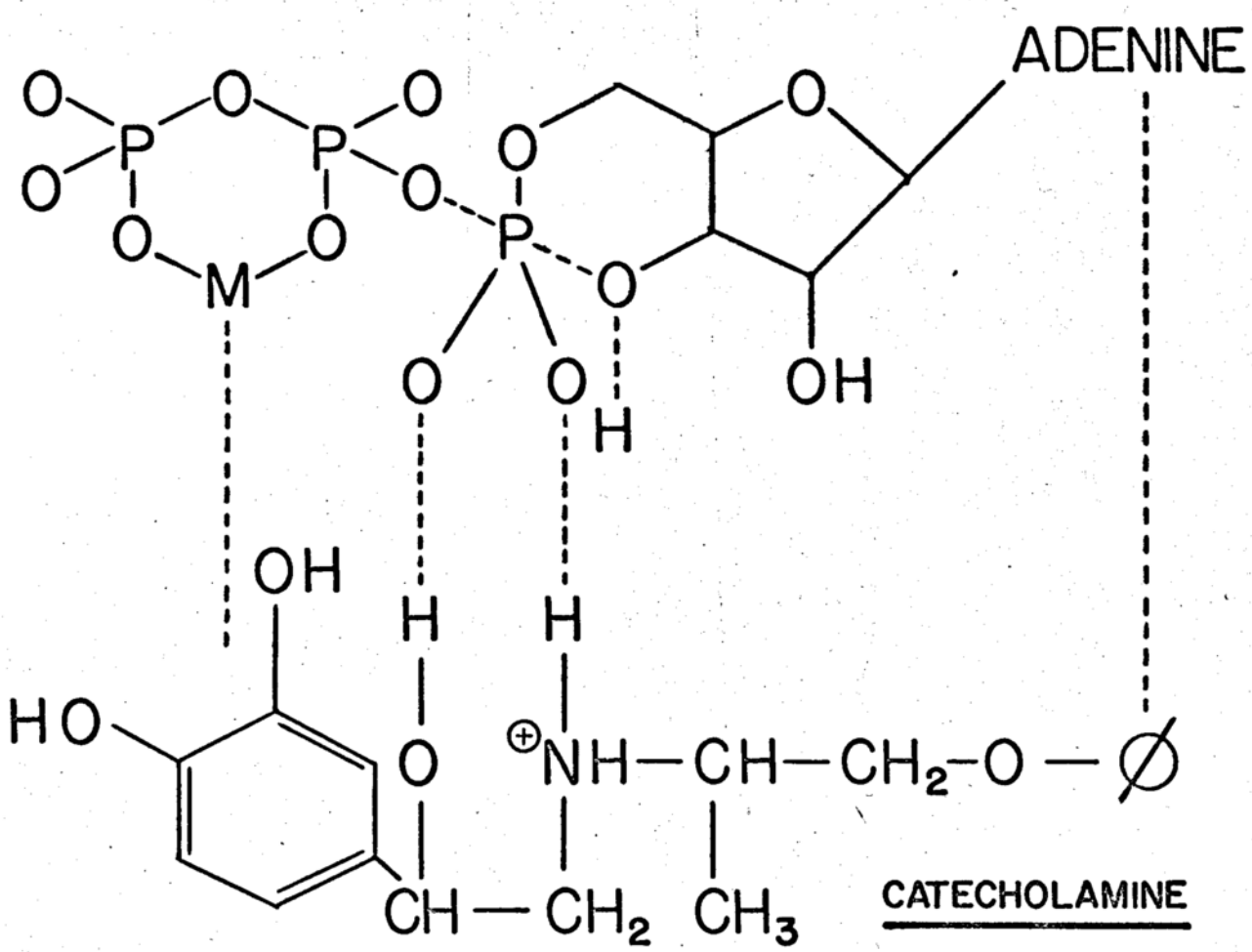
Barrett (132) found that the L(-) isopropyl arterenol isomer has double the affinity of the racemate and similarly D(+) isoxuprine, which represents the beta-mimetic component of its racemate (133), was also found to be more effective than the other stereoisomer (134). Wenke (135) and Page (136), in studies with both agonists and antagonists suggested that adipose tissue is a beta-trophic receptor and an alpha, beta receptor, respectively. Considering these facts, it is apparent that adipose tissue does not conform to a pure beta receptor system, but may be an adrenergic receptor of a single entity capable of responding to both types of stimulants.

These structure-activity relationships permit speculation as to the complementarity that exists between the drug molecule and the adipose tissue receptor system and serve to supplement views concerning characterization of this receptor. The attempts to define the nature of catecholamine and adrenergic receptor interaction in molecular terms has been formed partly by the knowledge of existing agonist and antagonist studies together with known chemical and enzymatic properties of functional groups on the agonist molecule. Belleau (137) has presented a possible mechanism for the catecholamine-adrenergic receptor interaction resulting in the conversion of ATP to 3',5'-cyclic-AMP. The concepts introduced were (1) that the nucleotide (ATP) was an integral part of the adrenergic receptor

surface, thus either in part or completely interacting with the catecholamine molecule, (2) that the catecholamine-induced conversion of ATP is facilitated by ion-pair formation between the ammonium ion (nitrogen atom is protonated at physiological pH) of the agonist with an anionic oxygen atom of the innermost phosphate of ATP, thereby permitting a nucleophilic attack by the 3'-hydroxyl of the ribose moiety and ejection of pyrophosphate with the formation of the cyclic nucleotide, and (3) assigning a metal chelation mechanism to the catechol ring in association with ATP. Based on molecular models, Belleau postulated that the catechol ring can be chelated to the oxygen atoms of the terminal phosphates of ATP and ion-pair formation can occur without the creation of stress in the complex formed. This model satisfies the assessment of optimal activity associated with the catechol group, since a non-catechol would less effectively chelate with a metal ion and thus possess less affinity for the ATP-bound enzyme surface. Although this hypothetical mechanism provided a significant understanding of the molecular interaction, it was necessary to dissociate the alpha and beta adrenergic systems. The following sequence of activity, isopropyl arterenol > epinephrine $\geq$ norepinephrine observed in adrenergic receptor systems required that the ion-pair formation scheme proposed above be modified. As was noted in this system, large substituents were tolerated at the amino nitrogen without a decrease in response and supports a recent hypothesis stating that there is a charge neutralization of the ammonium ion and the anionic ATP site rather than ion-pair formation. Belleau (138) has modified his initial mechanism to better coincide with the beta agonist-adenyl cyclase system to include: (1) the

overlap of large nonpolar (alkyl or aralkyl) substituents on the amino nitrogen interacting with the adenine ring of ATP, (2) the catalysis of ATP to 3',5'-cyclic-AMP as being facilitated by charge neutralization, and (3) the inclusion of the binding of the beta-hydroxyl group with the protein component of the enzyme-ATP complex.

Bloom and Goldman (139) recently presented a detailed elucidation of the interaction of catecholamines with adrenergic receptors based in part on the earlier ATP-adenyl cyclase model systems of Belleau. Their postulated molecular mechanism is discussed below with reference to the planar representation illustrated in Figure 16. These authors retained the Belleau concepts of the anchoring of the catechol ring by chelation and the charge neutralization of the 5'-phosphate moiety favoring displacement of pyrophosphate from the surface of the enzyme. However, they indicated a more important role for the beta-hydroxyl function. The neutralization of the anionic charge on the phosphate oxygen was suggested to lower the energy barrier for the attacking nucleophile (3'-hydroxyl on the ribose moiety). It is apparent that primary, secondary, and tertiary amines all are able to participate in the proposed interaction catalysing the breakdown of ATP. The activity obtained with the tertiary amine found in this study thus does not necessitate any modification of the proposed mechanism. The hydrogen bonding of the beta-hydroxyl group with the remaining anionic oxygen of the target phosphate is expected to promote the cyclization reaction in a manner analogous to that proposed for the ammonium ion. In this relationship, the removal of the hydroxyl function or its conversion to a ketone would effectively abolish the


NUCLEOTIDE
ADENINE
O
P
M
OH
H
HO
⊕NH—CH—CH2—O—∅
CH—CH2
CH3
CATECHOLAMINE

interaction with the anionic site and eliminate the participation of this group in the catalysis of ATP to 3',5'-cyclic-AMP. Decreased activity observed by alkylation of the alpha carbon fits well into this scheme since it is expected that this moiety may sterically hinder the precise alignment of the molecule at the target phosphate atom of ATP.

Thus, the results obtained with the adrenergic receptor system in adipose tissue are consistent with the mechanisms proposed for the interaction of catecholamine and ATP-bound adenyl cyclase enzyme. The complementarity of the phenethylamine molecule and adipose tissue receptor appears to be primarily associated with the binding forces of the chemical groups on the amino nitrogen, beta carbon, and the meta and/or para positions of the phenyl nucleus. It is apparent from the discussion above that the receptor area must allow for sufficient binding of these chemical groups to permit the approach of the molecule to the catalytic site for enzymatic conversion of ATP to 3',5'-cyclic-AMP as the initial step in catecholamine-induced lipolysis.

CONCLUSIONS

The chemical specificity shown by the adrenergic-adipose tissue system to exogenous substituted phenethylamines in the mobilization of free fatty acids (FFA) was determined. Small molecular modifications in chemical structure resulted in significant alterations of FFA mobilizing activities, thus indicating a high degree of complementarity of the agonists with receptors in adipose tissue in a manner similar to the interaction of substrate and enzyme. The dose-response and double reciprocal relationships obtained are consistent with an agonist-receptor interaction existent in adipose tissue suggesting that the measurement of mobilized FFA is proportional to the number of agonist-adipose tissue complexes formed.

A differentiation of the effectiveness of the agonists employed was shown by the calculation of the pharmacological parameters, intrinsic activity (efficacy) and affinity. These data have yielded the following conclusions relating chemical structure to biological activity:

1. Optimal activity of the agonist molecule was correlated with the presence of the catecholamine moiety. It appeared not to be obligatory for maximal release of FFA since several monohydroxylated phenethylamines (oxedrines) had significant mobilizing activity. The removal of either phenolic group from the catecholamine moiety markedly reduced the affinity of the agonist molecule in this adipose tissue system.

2. The beta-hydroxyl function appeared to play an important role in determining the affinity of the agonist molecule for the adipose tissue

system.

3. Alpha carbon alkylation decreased the affinity of the phenethylamine molecule in the presence of an ethyl substituent, but did not significantly alter mobilizing activity in the presence of a methyl substituent.

4. N-substitution of large alkyl or aralkyl groups considerably increased the affinity and enhanced the fat mobilizing activity of agonists employed. It is evident that potent FFA activity was associated with primary and secondary amines, and it was further noted that a tertiary amine possesses a much decreased affinity of the agonist molecule for this adipose tissue system.

5. Methylation of the meta-phenolic function, removal of the phenyl nucleus, or removal of the nitrogen atom from agonist molecules completely abolished FFA mobilizing activity.

Furthermore, from other information obtained in this study, it can be concluded:

1. Several compounds, whose action is mediated at least in part by a release of endogenous catecholamines, were inactive in this system. In addition, one of these compounds, tyramine, effectively antagonized norepinephrine-induced lipolysis.

2. The agonists metaraminol and octopamine, also known to possess an indirect action, were generally unaffected by prior reserpinization of the adipose tissue, suggesting that mobilization of FFA by these compounds is primarily related to a direct component of action in the adipose tissue system under study.

3. Although both alpha- and beta-type adrenergic stimulants were capable of mobilizing FFA from this adipose tissue system, it has been shown that the beta-type stimulants are much more active in this regard.

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Agonist-Receptor Interactions Involved with Mobilization of Free Fatty Acids from Adipose Tissue

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Numerous reports have indicated the increasing importance attributed to the mobilization of free fatty acids (FFA) as a contributing factor in certain disease states and as a primary energy source in normal body metabolism. It was the purpose of this study to define the structure-activity relationships inherent in the interaction of sympathomimetic drugs with the adrenergic receptor of epididymal fat tissue, in vitro, inasmuch as the sympathetic nervous system has been shown to exert a controlling influence over the mobilization of FFA in vivo.

Utilizing the rat epididymal fat pad as a source of biological tissue, the rates of FFA release were quantitated in the presence of various concentrations of sympathomimetic agonists. The data thus obtained were plotted in the standard dose-response relationship and the double reciprocal form to facilitate the calculation of the intrinsic activity (efficacy) and affinity constants. These two parameters were then employed to establish the structure-activity relationships.

It was established that structural modifications of the parent phenethylamine molecule produced marked changes in the affinity of the molecule for the adrenergic receptor tissue, and, to a much lesser degree, changes in the intrinsic activity possessed by the agonists.

Optimal activity was observed among the phenethylamines when the catechol moiety was present. The rank order of activity found for the catecholamines was protochylol > isopropyl arterenol > norepinephrine = epinephrine = nordefrin > ethylnorepinephrine > dopamine > adrenalone > N-methylepinephrine = phenisomone.

Small structural changes on the ethylamino side chain markedly altered the ability of the catecholamine to mobilize FFA. This phenomenon was most apparent when changes were made in the beta-hydroxyl function or substitution on the terminal nitrogen.

While optimal activity was possessed by the catecholamines, the results obtained with certain non-catecholamines indicated that the catechol nucleus was not obligatory for FFA mobilization. The presence of a hydroxyl group in either the para or the meta ring position was sufficient to produce maximal FFA mobilizing activity when the beta carbon of the side chain was also hydroxylated. It was clearly established that the beta hydroxyl group played an important role in the binding of the phenethylamines to the biological receptor tissue.

Similarly, N-substitution of a bulky alkyl or aralkyl group greatly enhanced the fat mobilizing activity of the phenethylamine molecule. In this regard, it was established that optimal activity was obtained with either primary or secondary amines. Tertiary amines, while still possessing maximal FFA mobilizing activity, were characterized as having a much decreased affinity for the adipose tissue receptor.

Methylation of the alpha carbon of the side chain did not markedly alter biological activity while ethylation in this position significantly decreased the affinity of the molecule. These results suggest that the

presence of substituents in the alpha position can sterically hinder the approach of the agonist molecule to the receptor area.

Compounds known to possess an indirect component of action in other adrenergic systems were studied in the rat epididymal fat pad system. Tyramine, amphetamine and ephedrine were completely inactive as agonists while octopamine and metaraminol were found to possess FFA mobilizing activity. Further investigation established that only those compounds possessing a significant degree of direct activity on the adrenergic receptor (metaraminol and octopamine) were capable of mobilizing FFA. Indeed, tyramine, while not capable of producing an agonistic effect in this system was found to be an antagonist of catecholamine-induced FFA mobilization.

The interaction between the phenethylamine molecule and the adipose tissue adrenergic receptor is believed to occur through a coupling of the agonist molecule with the ATP-adenyl cyclase system resulting in a conversion of the ATP to 3',5'-cyclic-AMP. The cyclic nucleotide, in turn, is believed to be responsible for the activation of lipolytic activity in adipose tissue. The structure-activity relationships established in this study are in agreement with the postulated structural requirements of Belleau and those of Bloom and Goldman for such an interaction.

Approved_____
Professor Kenneth F. Finger

Date_____