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ABSTRACT

Title of Dissertation: The regulation of blood flow and metabolism in adipose tissue: evidence for a role of adenosine

Sharon E. Martin, Doctor of Philosophy, 1984

Dissertation directed by: Emma L. Bockman, Ph.D., Associate Professor, Department of Physiology

Adenosine is proposed as a regulator of blood flow in unstimulated adipose tissue and in tissues in which lipolysis and vasodilation have been induced by the intravenous infusion of norepinephrine. In vitro studies have shown adenosine to stimulate glucose transport and to inhibit lipolysis. Experiments were designed to determine whether a cause-and-effect relationship exists between variations in tissue adenosine content and changes in vascular resistance, and whether adenosine regulates metabolism in vivo. Theophylline, an adenosine receptor antagonist, or adenosine deaminase, an enzyme which specifically degrades adenosine, was infused into subcutaneous adipose tissues of dogs in order to attenuate the actions of adenosine. Theophylline or adenosine deaminase was infused into both unstimulated tissues and tissues which later received an intravenous infusion of norepinephrine. Neither intraarterial nor intravenous theophylline antagonized the vascular effects of exogenous adenosine. Intravenous theophylline did not increase vascular resistance, decrease glucose uptake, or increase lipolysis in unstimulated tissues. No attenuation of the vasodilation caused by norepinephrine was observed. Theophylline did not appear to block the vascular or metabolic effects of endogenous adenosine. Adenosine deaminase significantly increased vascular resistance and decreased glucose uptake in unstimulated tissues. No stimulation of the basal lipolytic rate was observed. Adenosine deaminase completely

blocked the vasodilation caused by norepinephrine. Adenosine deaminase decreased, while norepinephrine increased, tissue adenosine content. A significant, inverse relationship between adenosine content and vascular resistance was observed. Lipolysis, as measured by glycerol release, was significantly correlated with tissue adenosine content and inversely correlated with vascular resistance. These studies support the concept of metabolic control of blood flow in adipose tissue and provide evidence for a role of adenosine as a regulator of vascular events. Adenosine also appears to regulate glucose uptake *in vivo*. However, in contrast to findings of *in vitro* studies, adenosine does not appear to inhibit lipolysis in adipose tissue *in vivo*.

THE REGULATION OF BLOOD FLOW AND METABOLISM IN ADIPOSE TISSUE:

Evidence for a role of adenosine.

by

Sharon E. Martin

Dissertation submitted to the Faculty of the Department of Physiology
Graduate Program of the Uniformed Services University of the Health Sciences
in partial fulfillment of the requirements for the degree of
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DEDICATION

In writing this dedication, I am thrown into memories of the four years I've spent attaining this goal. In nearly every flashback, three very special persons appear; persons without whom I would not have completed this facet of my training. Their love and their continued support have made all the difference. I thank them from the bottom of my heart.

I dedicate this work to:

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BACKGROUND

ADIPOSE TISSUE METABOLISM

Adipose tissue is the major site of energy storage in the body and can be thought of as an "energy buffer". The tissue alternately converts dietary fats into stored triglycerides and releases the stored triglycerides as free fatty acids. In the resting state, an estimated 50-70% of the body's total oxygen consumption is from the oxidation of free fatty acids (Carlson et al., 1963), which also constitute the major source of fuels for exercising skeletal muscle (Bulow and Madsen, 1976.)

Two major metabolic pathways are simultaneously active in adipose tissue: lipolysis and esterification (Figure 1). Lipolysis (the hydrolysis of the stored triglycerides to component free fatty acids and glycerol) elevates the plasma free fatty acids, ensuring the supply of fuel to the heart and skeletal muscles. Lipolysis involves the sequential breakdown of glyceroester bonds to yield three fatty acids and the glycerol backbone for every mole of triglyceride degraded. The pathway is catalyzed by a series of lipases. The first lipase, hormone-sensitive lipase, catalyzes the rate-limiting step, the removal of the first fatty acid (Newsholme and Start, 1973). Subsequent hydrolysis is then catalyzed by di- and monoglyceride lipases. The free fatty acids diffuse down their concentration gradient into the blood stream where they are transported bound to plasma proteins.

Esterification is the assembly of free fatty acids onto the glycerol backbone. The fatty acid components of these triglycerides are not formed de novo within the adipose tissue. Instead, they are derived either from fatty acids that are extracted from serum lipoproteins or from fatty acids that have been recently released during intracellular

lipolysis (Garratt et al., 1980). The reformation of triglycerides from recently liberated fatty acids is termed re-esterification.

Role of glucose

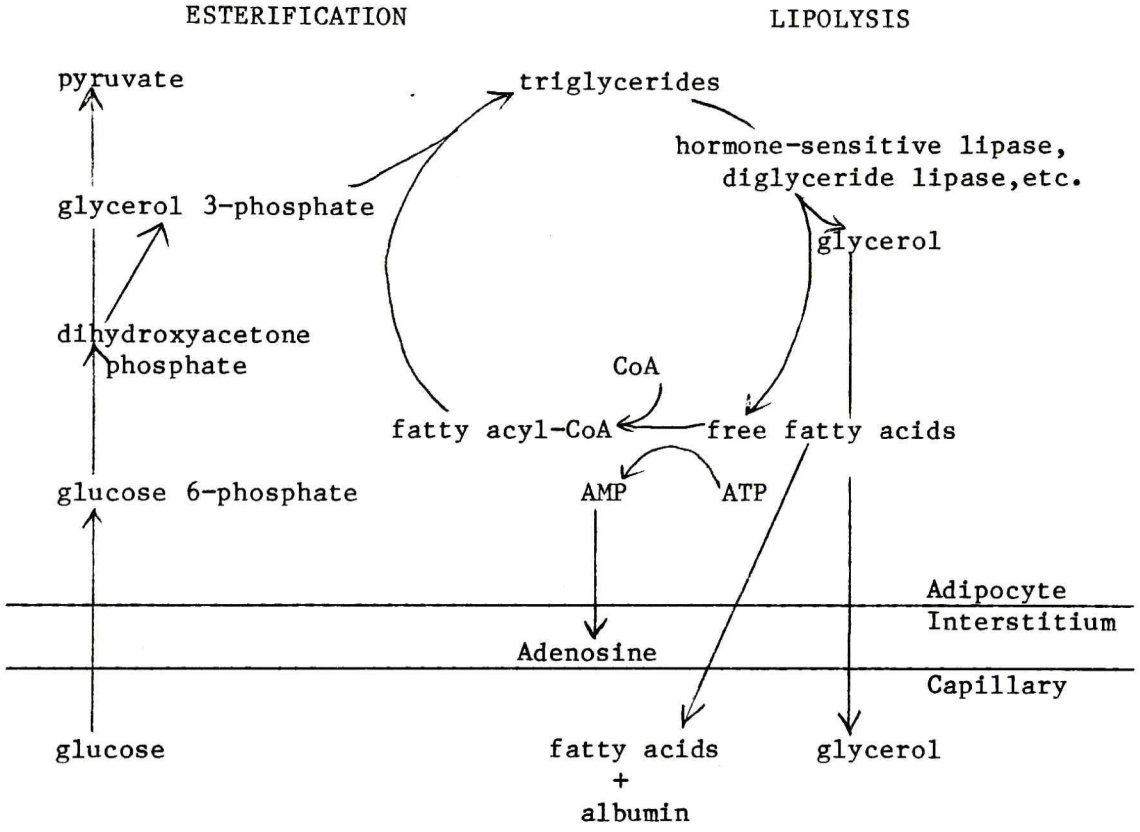
The rate of esterification is dependent on the intracellular supply of glucose and free fatty acids (Shapiro, 1963). High glucose concentrations provide a sparing action on fatty acid oxidation within the adipose tissue, allowing fatty acids to be channeled into pathways of re-esterification (Bally et al., 1960; Carlson et al., 1963). Glucose and other hexoses are critical for the maintenance of re-esterification for another reason: adipose tissue has a low activity of glycerol kinase which phosphorylates glycerol. Thus, glycerol cannot serve as a source of glycerol 3-phosphate which is required for esterification (Shapiro, 1963). The glycerol 3-phosphate is derived from dihydroxyacetone phosphate which is supplied through glycolysis (Figure 1).

Re-esterification is an energy-consuming pathway. During re-esterification, every mole of free fatty acid that is to be joined to a glycerol backbone must be "activated". Activation is the process in which a coenzyme A (CoA) group is attached to the free fatty acid, yielding fatty acyl-CoA. This step requires the breakdown of one mole of adenosine triphosphate (ATP) to adenosine monophosphate (AMP) per mole of free fatty acid activated (Figure 1).

During complete lipolysis, the free fatty acids and glycerol in a ratio of 3 to 1 are liberated for diffusion into the blood stream. In reality, complete hydrolysis does not occur, and some of the liberated fatty acids are re-esterified into triglycerides. Thus, neither fatty acid nor glycerol release is a completely accurate index of lipolysis. However, since the glycerol that is liberated during lipolysis cannot

be salvaged for re-esterification, glycerol release is a generally accepted index of lipolysis (Vaughan and Steinberg, 1963).

Figure 1. Pathways of adipose tissue metabolism



(Adapted from Newsholme and Start, 1973)

In vitro studies have shown that maximal lipolytic stimulation leads to an intracellular buildup of free fatty acids and a decrease in tissue ATP levels (Angel et al., 1971; Bihler and Jeanrenaud, 1970). High intracellular levels of free fatty acids may uncouple oxidative phosphorylation (Bihler and Jeanrenaud, 1970; Fain, 1979) and are feedback inhibitors of lipolysis (Burns et al., 1978). A buildup of intracellular free fatty acids could be prevented either by assuring re-es-

terification by the availability of glucose (Bally et al., 1965) or by trapping the free fatty acids outside the cell by the presence of sufficient albumin (Angel et al., 1971; Rodbell, 1965; Sjöstrom et al., 1977).

The importance of an adequate glucose supply in the maintenance of lipolysis is supported by in vitro studies. High glucose concentrations per se promote lipogenesis and fat storage in unstimulated (basal) adipocytes (Jeanrenaud and Renold, 1959). However, glucose can increase the magnitude of lipolysis in catecholamine-stimulated adipocytes (Jungas and Ball, 1963). Addition of glucose to lipolytically stimulated adipocytes actually enhanced oxygen consumption and the rate of glycerol release. The authors suggested that intracellular free fatty acids were re-esterified, thereby preventing them from reaching inhibitory levels (Bally et al., 1965; Jeanrenaud and Bihler, 1963). If lipolytic stimulation of isolated adipocytes is prolonged, the glycerol release declines over time in the absence of glucose, but the rate of decline is decreased when glucose is added to the medium (Knight and Iliffe, 1973). If glucose uptake is necessary to maintain the lipolytic rate, an energy expenditure by the tissue is required. This energy cost may appear wasteful. However, the utilization of ATP in re-esterification permits continued lipolysis and the subsequent release of fuel into the blood stream. Additionally, limiting the rise of intracellular free fatty acids prevents the possible inhibition of oxidative phosphorylation.

Role of albumin

The presence of albumin in the medium bathing isolated adipocytes has been shown to influence the lipolytic rate (Angel et al., 1971; Sjöstrom et al., 1977). Norepinephrine increased glycerol release to

a greater degree when sufficient albumin was available (Sjöstrom et al., 1977). Sjöstrom et al. (1977) reported that the flow rate of medium perifusing isolated adipocytes was directly related to the glycerol release. A likely explanation is that the increase in delivery of albumin allowed increased removal of free fatty acids and the subsequent increase in lipolysis as measured by glycerol release. In vitro studies of Scow et al. (1965) showed that fatty acid release was greater from fat pads of rats that were perifused with a medium containing high amounts of albumin than was release from pads perifused with a medium containing low amounts of albumin. In vivo studies have also shown that the removal of free fatty acids promotes lipolysis. Bülow and Madsen (1981) increased the delivery of albumin to isolated fat pads of dogs by increasing the concentration of albumin in the blood or by lncreasing the blood flow. In both situations, they reported an enhanced lipolytic rate. They also showed that a decrease in blood flow caused an increased fatty acid:albumin ratio and a decrease in fatty acid mobilization. They concluded that the presence of sufficient carrier for the fatty acids was essential to maintain the lipolytic rate. In exercising dogs, in which sympathetic nervous activity triggered lipolysis, Bülow (1982) has shown that the release of fatty acids and of glycerol parallels the increase in adipose tissue blood flow. Also, as fatty acid mobilization increases, so does the calculated rate of re-esterification [(3 x glycerol production) - FFA production] (Bülow, 1982). These findings support an hypothesis that the lipolytic rate of adipose tissue is dependent on the removal of intracellular free fatty acids. By increasing the blood flow through adipose tissue, more albumin can be delivered to the tissue, thereby promoting the

diffusion of free fatty acids from the tissue.

ASSOCIATION OF METABOLISM AND BLOOD FLOW

The interrelationship between adipose tissue metabolism and blood flow has been observed by many investigators. Many lipolytic agents, such as adrenocorticotrophic hormone (ACTH) and glucagon, cause vasodilation in adipose tissue (Engel and White, 1960; Lewis and Matthews, 1970). Intravenous administration of norepinephrine to human subjects significantly increased fatty acid mobilization and adipose tissue blood flow (Nielsen et al., 1968). Lipolysis is stimulated in diabetic rats and is accompanied by increases in blood flow through adipose tissue (Lucas and Foy, 1977). These findings indicate that an increase in lipolysis is accompanied by elevations in adipose tissue blood flow. The rate of circulation may be linked to the level of lipolysis as proposed by Nielsen et al. (1968) and McCoy (1980).

Vascular responses to norepinephrine

In physiologic situations, the primary stimulus for lipolysis is norepinephrine, a catecholamine released by sympathetic nerves and the adrenal medulla. Once released, norepinephrine acts at adrenergic receptors of two types: alpha- and beta-receptors. Beta-receptors are classified into two categories: $beta_1$- and $beta_2$-receptors. $Beta_1$-receptors mediate metabolic effects, such as increased glycogenolysis and increased lipolysis. $Beta_2$-receptors mediate vasodilation. Alpha-receptor activation causes vasoconstriction. Norepinephrine has actions primarily on the alpha- (vasoconstriction) and $beta_1$- (lipolysis) receptors, with minimal action on the $beta_2$-receptors (Rosell and Belfrage, 1979).

In most vascular beds, norepinephrine causes vasoconstriction (Hoffbrand and Forsyth, 1973). However, in adipose tissue, the vascular effects of norepinephrine are variable and complex. Brief (4 minute) stimulation of the adrenergic nerve supplying canine subcutaneous adipose tissue at physiologic frequencies causes release of norepinephrine and vasoconstriction (Ballard and Rosell, 1971; Ngai et al., 1966). With prolonged stimulation (30 minutes) or stimulation at higher frequencies, the vasoconstriction converts to a vasodilation (autoregulatory escape) even though stimulation continues (Rosell, 1966). The autoregulatory escape can be diminished by the administration of a beta-receptor blocker, propranolol (Belfrage, 1978a). The administration of propranolol also blocks the lipolytic response. Autoregulatory escape can be potentiated with the administration of an alpha-receptor blocker, dihydroergotamine (Ngai et al., 1966). These findings indicate that brief stimulation at low frequencies causes alpha-receptor activation (vasoconstriction). At higher frequencies or stimulation for longer periods of time, the alpha-mediated vasoconstriction occurs, but it is overridden by beta-mediated vasodilation (autoregulatory escape). However, as discussed below, whether the beta-effects are direct on the vascular smooth muscle or indirect through metabolism remains controversial.

Intravascular infusion of norepinephrine gives equally variable responses. Intraarterial infusion of norepinephrine into subcutaneous adipose tissue of dogs caused a concentration-dependent vasoconstriction (Ballard et al., 1971). Unlike the results with nerve stimulation, if vasoconstriction occurred, only rarely did it change to vasodilation. Norepinephrine caused a net increase in glycerol release, indicating that intraarterial norepinephrine stimulated lipolysis.

In attempts to mimic more closely the effects of circulating catecholamines, Ballard (1973) and Hoffbrand and Forsyth (1973) administered norepinephrine intravenously to dogs and monkeys, respectively. Ballard (1973) reported that the intravenous administration of norepinephrine caused vasodilation in adipose tissue. Hoffbrand and Forsyth (1973) found that subcutaneous adipose tissue was the only tissue to respond with vasodilation. Vascular resistance to the heart and brain did not change, while it increased in all other tissues studied. When subcutaneous adipose tissue of dogs was treated with an alpha-blocker, dihydroergotamine, the vasodilation was enhanced (Ballard, 1973; Oro et al., 1965). Beta-receptor blockade with propranolol abolished not only the vasodilator responses (Ballard, 1973; Hjemdahl and Fredholm, 1976; Ngai et al., 1966) but also the increases in lipolysis (Ballard, 1973; Fredholm and Karlsson, 1970). Beta-receptor blockade prolonged and potentiated the constrictor response to intraarterially administered norepinephrine (Belfrage, 1978a). These findings indicate that both the lipolytic response and the vasodilation that occur after the intravenous administration of norepinephrine are beta-receptor mediated events. In these studies, blockade of one response was not achieved without blockade of the other.

Theories for the vascular effects of norepinephrine

Direct, receptor-mediated vasodilation

Although agreement exists among investigators that increases in lipolysis occur simultaneously with increases in adipose tissue blood flow, two major theories have been proposed to explain the relationship between the two events and the discrepancies in the vascular effects of norepinephrine. Rosell and Belfrage (1979) propose that two groups

of receptors are stimulated by catecholamines. They suggest that the vasoconstriction that occurs during nerve stimulation is mediated by alpha-receptors whose anatomic location make them more easily accessible to norepinephrine released from nerve terminals ("innervated" receptors). In contrast, intravenously infused norepinephrine causes stimulation of beta-receptors that are in better anatomic location to respond to circulating catecholamines than to those released from nerve terminals (Ballard, 1973). These receptors have been classified as "humoral" or "non-innervated" (Ballard, 1978; Belfrage, 1978a; Rosell and Belfrage, 1979). These authors also propose that the vasodilation that occurs during prolonged nerve stimulation reflects the overflow of high concentrations of norepinephrine. Norepinephrine released from nerve terminals at the adventitio-medial border would be in sufficiently large quantities to diffuse and interact with the "noninnervated, humoral" beta-receptors in the media of the blood vessels. Discrepancies in this theory become evident when considering the vascular response to intraarterially administered norepinephrine. Arterial infusion of norepinephrine would be expected to interact with the humoral (vasodilatory) receptors. However, the intraarterial administration of norepinephrine causes vasoconstriction, as is seen with sympathetic nerve stimulation (Ballard, 1973). Ballard (1973) has attributed this discrepant finding to differences in the concentration of circulating catecholamines that are achieved by the two methods of infusion. Intraarterial infusion leads to a more rapid increase in the plasma concentration of norepinephrine, leading to vasoconstriction. In contrast, she proposed that a slower increase in concentration occurs with intravenous infusion, which in some way elicits vasodilation.

Additional aspects of this theory come from Belfrage's studies (1978a, 1978b) of the adrenergic receptors in skeletal muscle and adipose tissue. Administration of $beta_2$-selective agonists caused a greater vasodilation in skeletal muscle than in adipose tissue. Conversely, $beta_1$-selective ("metabolic") agonists were unable to dilate blood vessels in skeletal muscle but did cause vasodilation in adipose tissue. Norepinephrine was a more potent vasoconstrictor in skeletal muscle than in adipose tissue. Belfrage (1978a) also showed that $beta_1$-receptor blockade in adipose tissue abolished both the lipolytic and vascular responses to norepinephrine while a $beta_2$-receptor blocker failed to alter either response. Belfrage (1978a, 1978b) concluded that, in adipose tissue, the vascular and metabolic responses to norepinephrine were mediated by a $beta_1$-receptor. He proposed that the vasodilation is elicited by a direct action of norepinephrine on the vascular smooth muscle. This effect of norepinephrine contrasts with effects in other organs, such as skeletal muscle, in which the receptors mediating vasodilation are $beta_2$-receptors and are unaffected by norepinephrine (Belfrage, 1978b). Nevertheless, it has been proposed that the lipolytic response and the vascular response are direct actions of norepinephrine on $beta_1$-receptors on the adipocyte and on the vascular smooth muscle, respectively (Ballard, 1978; Belfrage, 1978a; Belfrage, 1978b; Rosell and Belfrage, 1979).

As indicated above, the theory of a direct, vasodilatory action of norepinephrine on vascular smooth muscle fails to account for the vasoconstriction that is seen when norepinephrine is infused intra-arterially; particularly if the "humoral" receptors are those that norepinephrine contacts first and if the "humoral" receptors mediate vaso-

dilation. This theory also addresses only the vasodilation caused by norepinephrine. Other lipolytic agents not affecting adrenergic receptors cause vasodilation (Lewis and Matthews, 1970). To prove more conclusively that the vascular and metabolic effects of norepinephrine are separate phenomena, one should be able to separate these effects. In Belfrage's studies, beta-receptor blockade not only blocked the vascular effects but also blocked the change in metabolism.

Indirect, metabolically mediated vasodilation

A second theory can be proposed to explain the relationship between adipose tissue metabolism and blood flow. In in vivo studies, Nielsen et al. (1968) showed a parallel relationship between increased lipolysis and increased blood flow. They concluded that the vasodilation that occurred with the intravenous administration of norepinephrine was a secondary, indirect effect of norepinephrine related to increases in the release of free fatty acids. In studies of Lewis and Matthews (1970), extracts of lipolytically stimulated tissues caused vasodilation when infused into unstimulated (basal) tissues whereas extracts of unstimulated tissues did not. They suggested that the increase in adipose tissue blood flow that occurred after administration of many lipolytic agents was functional, i.e. related to the metabolism of the tissue. These findings all indicate that the metabolic rate may determine the blood flow through the tissue.

The "metabolic" theory can explain the discrepancies of the reponses to the intravascular infusion of norepinephrine. In contrast to the effects of intravenous, systemic administration of norepinephrine, the intraarterial infusion has limited systemic actions and limited, if any, liver glycogenolysis occurs. The intraarterial infu-

sion would also activate the vascular alpha-receptors and cause vasoconstriction. The vasoconstriction, in concert with the limited rise in plasma glucose, would limit the washout of free fatty acids (albumin) from the tissue. Lipolytic stimulation would occur, evidenced by the increased glycerol release (Ballard et al., 1971), but the activity would be limited by the rapid accumulation of lipolytic products. The intracellular level of free fatty acids would increase without the protective effects of re-esterification and inhibit general metabolic processes. The metabolic rate would then decline. Any vasodilation related to the level of metabolism would not be sufficient to override the direct alpha-mediated vasoconstriction. Intravenous norepinephrine, on the other hand, stimulates a rise in plasma glucose (Ballard, 1973). The increase in glucose utilization by the adipose tissue would promote re-esterification and continued lipolysis. Consistent vasodilation in response to intravenous norepinephrine occurred at the time of significant increases in plasma glucose concentration (Ballard, 1973). At lower infusion rates of norepinephrine, no consistent vasodilation nor increases in plasma glucose were seen. These data provide indirect support for the hypothesis that metabolic and vascular events are linked.

To support either the "direct" or the "metabolic" theory, separation of the vascular and metabolic events becomes necessary. Earlier studies (Ballard and Rosell, 1971; Belfrage, 1978a; Ngai et al., 1966) that used beta-blockade abolished both the vasodilation and the lipolytic response to nerve stimulation. From these studies, conclusions about the relationship between adipose tissue metabolism and blood flow cannot be drawn. If norepinephrine acts directly at the vascular smooth muscle to cause vasodilation, inhibition of adipose

tissue metabolism should not affect the dilatory response. Mjös and Akre (1971) first attempted to separate the two phenomena by the administration of nicotinic acid, an inhibitor of lipolysis. The administration of norepinephrine caused an increase in adipose tissue blood flow and lipolysis. In the presence of nicotinic acid, norepinephrine caused similar increases in blood flow while the lipolytic response was blocked. They concluded that norepinephrine directly stimulated the vascular smooth muscle and that the increase in blood flow was not related to a secondary increase in metabolism. However, the arterial blood pressure was also increased by the administration of norepinephrine, allowing for the possibility that the blood flow was increased simply because the blood pressure had increased. Mjös and Akre (1971) had not elicited vasodilation with norepinephrine, even in the absence of nicotinic acid. Conclusions about the mechanism of norepinephrine to vasodilate cannot be made from this study.

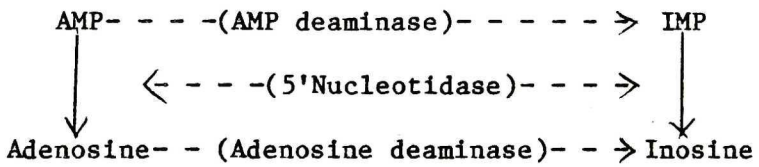
Bülow (1981) also studied the two phenomena in humans performing an exercise protocol. In the absence of nicotinic acid, exercise increased adipose tissue blood flow and lipolysis. In the presence of nicotinic acid, neither the lipolytic response nor the vasodilation occurred. Bülow's study indicates that when sympathetic stimulation occurs during exercise, if the lipolytic response is blocked by nicotinic acid, adipose tissue blood flow does not increase. If norepinephrine causes vasodilation by a direct action on the vascular smooth muscle, vascular resistance should decrease to a similar degree in the presence as well as in the absence of nicotinic acid. Recently, Madsen and Malchow-Möller (1983) inhibited lipolysis in rat adipose tissue by the administration of nicotinic acid and reported a 28% decrease in adipose

tissue blood flow. Arterial blood pressure was not affected so that the decrease in blood flow was due to an increased vascular resistance. The latter two studies clearly support the hypothesis that adipose tissue blood flow is a function of metabolism and that norepinephrine dilates by an indirect action related to the level of metabolic stimulation.

If the level of metabolism is related to the rate of circulation through adipose tissue, it makes sense that at a particular level of metabolic stimulation a signal is triggered to elicit vasodilation. In this manner, regardless of the lipolytic stimulus, vasodilation would occur once a critical point in metabolism is reached. This point may be related to the concentration of intracellular free fatty acids. If increases in adipose tissue metabolism led to the production of a substance that causes vasodilation, the delivery of substrates and the removal of the lipolytic products would progress at a rate in keeping with the degree of lipolytic stimulation. It is possible that individual lipolytic agents could elicit vasodilation by separate, direct actions at the vasculature. Alternately, individual agents could cause increases in blood flow if, through variations in the degree of metabolism, changes in the level of a vasodilator occurred. This "local control" of blood flow has been proposed to exist in many tissues, including skeletal and cardiac muscles (Berne et al., 1979; Olsson, 1981). Berne's hypothesis is that when cardiac metabolism is increased, the tissue PO_2 falls as tissue energy stores are depleted. As the tissue PO_2 falls, a vasodilator metabolite is produced. The metabolite would then diffuse into the interstitial space and cause relaxation of the vascular smooth muscle. Tissue blood flow and tissue oxygenation would be restored.

Unlike the situation in cardiac muscle, the oxygen content may not be the sensed variable in adipose tissue. Although stimulation of the sympathetic nerve does increase metabolism and increase oxygen consumption (Fredholm et al., 1976; Mjös and Akre, 1971), McCoy (1980) found only a weak correlation between oxygen consumption and blood flow. In adipose tissue, the level of intracellular free fatty acids may be the most closely sensed and regulated variable, evidenced by the fact that tissue ATP stores diminish in proportion with the rise in intracellular free fatty acids (Bihler and Jeanrenaud, 1970; Fain, 1979).

As intracellular free fatty acids accumulate, re-esterification is stimulated. Therefore, changes in free fatty acids indirectly signify energy use by the tissue. These changes could indirectly initiate changes in blood flow. Re-esterification would limit the buildup of intracellular fatty acids and also provide the substrate for the production of a potent vasodilator, adenosine. As re-esterification proceeds, ATP would be degraded to AMP as each fatty acid is activated. Adenosine monophosphate can be degraded by two routes, as seen in the following diagram:



Adenosine monophosphate can be deaminated to inosine monophosphate (IMP) by the action of AMP deaminase. Adenosine monophosphate can also be dephosphorylated to the nucleoside, adenosine, by the action of 5'-nucleotidase. 5'-Nucleotidase also catalyzes the conversion of IMP to inosine. As more high energy phosphates are used by the tissue,

more AMP would be produced, increasing the potential for adenosine production. Through adenosine production, the increases in tissue metabolism could lead to proportional increases in blood flow.

ADENOSINE AS A MEDIATOR

Production by adipose tissue

If adenosine is proposed as a mediator of the vascular responses seen *in vivo*, adipocytes must be shown to produce adenosine both in the basal and in the stimulated state. Additionally, a significant pathway of AMP degradation to adenosine rather than to IMP should exist. The 5'-nucleotidase activity has been located at the plasma membrane of rat adipocytes (Newby *et al.*, 1975). Other enzymes involved in AMP degradation have not been measured in rat adipocytes. No analysis of these enzymatic pathways in adipose tissue of dogs has been made to date.

In vitro studies have shown that, in the basal state, adipocytes release substantial quantities of adenosine. Over time, cellular ATP content decreases and up to 0.2 nmoles adenosine/10^5 cells/20 minutes are released (Schwabe *et al.*, 1973). When isolated adipocytes were lipolytically stimulated, Schwabe *et al.* (1973) were unable to show that adenosine release was increased. Fain (1979) showed norepinephrine to increase the release of AMP but not adenosine. These results are contradictory to the local control hypothesis. However, the assay for adenosine used by Schwabe *et al.* (1973) was not of sufficient sensitivity to determine whether or not adenosine was released. Moreno *et al.* (1979) reported that, in Fain's studies, the medium contained bovine serum albumin that was contaminated with adenosine deaminase, so that any adenosine would have been rapidly degraded. These technical difficulties detract from conclusions made from the aforementioned studies. More

recently, in support of the local control theory, Fain and Malbon (1979) showed norepinephrine to increase the amount of adenosine released from isolated adipocytes.

In vivo, in unstimulated adipose tissue, adenosine is continually released (Fredholm and Sollevi, 1981). Administration of norepinephrine into and sympathetic stimulation of isolated, perfused fat pads of dogs increased the rate of adenosine outflow into the venous blood (Fredholm and Sollevi, 1981; Sollevi and Fredholm, 1983). McCoy (1980) measured the tissue content of adenosine in fat pads of dogs and reported that the intravenous administration of norepinephrine and glucose tended to increase the tissue content of adenosine. These studies all indicate that adipose tissue has the capacity to produce adenosine in the basal state and that adenosine production is enhanced during lipolytic stimulation.

Regulation of blood flow

In keeping with the local control theory, increases in adenosine should cause increases in blood flow. Vasodilation can be induced in canine adipose tissue by amounts of adenosine as low as 10^{-10} moles (Sollevi and Fredholm, 1981a). A doubling of blood flow is seen at an arterial adenosine concentration of 0.5 μM (Sollevi and Fredholm, 1981a). McCoy (1980) showed the tissue content of adenosine to be inversely correlated with vascular resistance in adipose tissue. When the endogenous adenosine concentration is increased by the administration of drugs that prevent the degradation or uptake of adenosine (erythro-9-2-hydroxy-3-nonyladenine (EHNA) and dipyridamole, respectively), "basal" (resting) blood flow is increased (Sollevi and Fredholm, 1981a; Sollevi et al., 1981; Sollevi and Fredholm, 1983). Treatment of the tissue

with theophylline, an adenosine receptor blocker, caused a 40-50% decrease in "basal" blood flow (Sollevi and Fredholm, 1981a; Sollevi et al., 1981). The ability of theophylline to block endogenous adenosine was tested by comparison of the vasodilation with adenosine administered before and after theophylline. Theophylline decreased the potency of adenosine by one order of magnitude (Sollevi and Fredholm, 1981a).

Although these studies all appear to indicate that adenosine regulates blood flow in basal, unstimulated adipose tissue, certain technical problems make accurate conclusions from these studies difficult. Sollevi and Fredholm (1981a) studied the effects of theophylline in tissues that were perfused at constant flow. The constant-flow perfusion may be limiting in terms of delivery of substrates to and removal of lipolytic products from the tissue. If the basal lipolytic rate is high in a particular animal, the limited blood flow may impair the metabolic performance so that theophylline treatment would yield variable results. The ranges of resting blood flows among the in vivo studies were different. Sollevi and Fredholm (1981a) and Sollevi et al. (1981) reported a wide range (2 to 23 ml/min/100g) of blood flow. Although adipose tissue is characteristically variable in blood flow and metabolism, an extremely large range of resting blood flow may indicate that the preparations are not at similar metabolic or hemodynamic states, making comparisons among preparations inappropriate.

The method of calculating the vasodilator response to adenosine before and after theophylline administration is unclear. Sollevi and Fredholm (1981a) reported the response as a percent increase in "basal" (resting) blood flow. Whether "basal" in their studies means the blood flow immediately preceding the administration of adenosine or the

blood flow at the onset of the experiment was not stated. Because blood flow decreases with time and because theophylline is reported to further decrease the baseline flow (Sollevi and Fredholm, 1981a), the method of calculating the adenosine response is critical to accurate interpretation of the ability of theophylline to block the effects of adenosine. These in vivo findings support a role of adenosine in the regulation of blood flow to unstimulated tissues, but because of technical variations among and within studies, additional evidence is needed before firm conclusions can be made.

Adenosine may also mediate the vascular responses to prolonged but not to brief sympathetic stimulation. During brief sympathetic stimulation, blood flow is dramatically reduced. When the stimulation is discontinued, a pronounced overshoot of blood flow (hyperemia) occurs. This hyperemia is termed "reactive" in that it is in response to an insult to the tissue, a severe vasoconstriction. The vasoconstriction may seriously limit the oxygen supply to the tissue, and the developing hypoxia may trigger the production of many vasoactive agents. With prolonged stimulation, after an initial decrease, blood flow increases toward pre-stimulatory values (autoregulatory escape). Reactive hyperemia occurs when the prolonged stimulation is discontinued. If the endogenous adenosine concentration is increased by the administration of dipyridamole and EHNA, neither the initial vasoconstriction nor the hyperemic response to brief stimulation is altered (Sollevi et al., 1981). Brief sympathetic stimulation has not been shown to increase the outflow of adenosine from the tissue (Sollevi and Fredholm, 1981b). Thus, the administration of these agents would not be expected to influence the vascular response. The autoregulatory escape, but not the

peak hyperemic response, to prolonged nerve stimulation was enhanced by dipyridamole and EHNA (Sollevi and Fredholm, 1983). Theophylline caused a decrease in the autoregulatory escape but did not affect the poststimulatory hyperemia of prolonged nerve stimulation (Sollevi and Fredholm, 1983). Sollevi and Fredholm (1983) concluded that adenosine mediates the autoregulatory response but not the poststimulatory hyperemia of prolonged nerve stimulation because dipyridamole, EHNA, and theophylline did not alter the poststimulatory hyperemic response. However, if after nerve stimulation, adenosine release in control tissues is sufficient to cause maximal dilation, increasing the endogenous concentration of adenosine would have no additional effect. Theophylline is a competitive blocker (Wolff et al., 1981) so that after stimulation, the endogenous concentration of adenosine may have increased enough to override the theophylline blockade. Failure of these drugs to alter the poststimulatory hyperemia does not necessarily mean that adenosine plays no role in mediating the hyperemia.

Association of metabolic and vascular events

The above studies examined the role of adenosine in the regulation of blood flow to unstimulated tissues and in the reactive hyperemia that follows nerve stimulation. To date, only McCoy's studies (1980) have investigated the relationship between adenosine and the hyperemia caused by the intravenous administration of norepinephrine. This hyperemia is not preceded by a severe vasoconstriction and can be called "active" or "functional" in that blood flow increases as the metabolic work of the tissue increases. This situation is analagous to that seen in exercising skeletal or cardiac muscle. In active hyperemia, the signal for the increased blood flow is an intracellular one related to

the degree of metabolic demand as opposed to a limitation of substrate delivery or a washout of metabolic products as is seen in reactive hyperemia. The production of a vasodilator metabolite is related to the degree of metabolic stimulation rather than to hypoxia or a deficiency in substrate. The vascular response to intravenous norepinephrine differs from that of nerve stimulation in that the vasoconstriction is moderate, wanes with time, and blood flow increases above pre-norepinephrine levels. During nerve stimulation, the blood flow is markedly reduced and then increases toward but does not reach control blood flow. McCoy (1980) found that the intravenous infusion of norepinephrine increased both blood flow and the concentration of adenosine in adipose tissue. Over a wide range of vascular resistances, tissue adenosine content correlated inversely with vascular resistance.

In addition, McCoy (1980) examined the relationship of the metabolic state of the tissue to the vascular resistance and to the tissue adenosine content. As lipolysis increased (as measured by the increased free fatty acid and glycerol release), vascular resistance decreased. In addition, vascular resistance correlated inversely with indices of lipolysis (fatty acid and glycerol releases) indicating a relationship between the degree of lipolytic stimulation and the change in blood flow through the tissue. Glucose uptake was also positively correlated with tissue adenosine content. Since glucose is necessary to maintain re-esterification and since re-esterification is the main energy-consuming path, these findings support the hypothesis that as the energy consumption of the tissue increases, the production of a vasodilator metabolite occurs. Specifically, the metabolic theory proposes that re-esterification would lead to increased tissue levels

of AMP. As more adenosine is formed from the rising levels of AMP, the adenosine could diffuse into the interstitium and cause vasodilation. The vasodilation would maintain the integrity of the tissue during the increased metabolism. McCoy (1980) showed that the degree of metabolic stimulation parallels and may direct changes in blood flow through the increased production of the vasodilator, adenosine. These findings are the first strong evidence for the local (metabolic) control hypothesis and indicate that adenosine may be a mediator of the vascular responses to intravenous norepinephrine.

Regulation of metabolism

Feedback inhibition of lipolysis

If adenosine could also regulate the metabolic rate of the tissue in combination with vasodilation, tissue function could be finely balanced and maintained. In 1973, Ebert and Schwabe reported that adenosine increased the lipolytic rate in unstimulated, isolated adipocytes. However, other studies (Dietrich and Schwabe, 1975; Ohisalo, 1981; Turpin et al., 1977) reported that the removal of adenosine by the administration of adenosine deaminase caused an increase in the basal lipolytic rate. Turpin et al. (1977) reported that the addition of adenosine to unstimulated, isolated rat adipocytes had no effect on the basal lipolytic rate. Their findings led them to conclude that the endogenous release of adenosine was sufficient to inhibit unstimulated lipolysis maximally. Ohisalo (1981) was also unable to show an effect of exogenous adenosine on the basal lipolytic rate. Although this finding is in keeping with that of Turpin et al. (1977), one drawback was evident. In Ohisalo's study (1981), lipolysis was generally low and at the limits of detection. Therefore, changes induced by adenosine,

either to inhibit or to stimulate lipolysis, may not have been detected.

Schwabe et al. (1973) found that the concentration of isolated cells greatly influenced the magnitude of hormone-stimulated lipolysis. Dilute cell suspensions could be stimulated to a much greater degree than could a suspension of a large number of cells. Schwabe et al. (1973) reported the accumulation of adenosine in concentrated cell suspensions and the subsequent reduction in the level of cyclic AMP. As the cyclic AMP level dropped, glycerol release was inhibited. In dilute cell suspensions, the amount of adenosine that accumulated was not sufficient to inhibit the effect of catecholamines on the cyclic AMP levels. Their findings initiated many investigations into the role of adenosine as a feedback regulator of lipolysis. Micromolar concentrations of adenosine are able to inhibit cyclic AMP accumulation and glycerol release induced by low concentrations of catecholamines (Dietrich and Schwabe, 1975; Ebert and Schwabe, 1973; Fain, 1973; Fredholm, 1978; Ohisalo, 1981). Turpin et al. (1977) found that the removal of endogenous adenosine by the administration of adenosine deaminase potentiated the lipolytic response to epinephrine; 180% of the response to epinephrine alone was achieved. However, Dietrich and Schwabe (1975) reported that adenosine deaminase did not have additive effects with lipolytic agents. In these studies (Dietrich and Schwabe, 1975), the catecholamine concentration was 3 μM, a maximal stimulatory concentration. Adenosine is unable to inhibit lipolysis at a 3 μM concentration of catecholamines (Ebert and Schwabe, 1973). Therefore, the addition of adenosine deaminase would not be expected to potentiate the lipolytic response. These studies clearly indicate that adenosine is capable of attenuating the lipolytic effects of catecholamines in adipose tissue in vitro. In

keeping with the local control theory, adenosine would reduce the magnitude of lipolysis and prevent the accumulation of products that are potentially inhibitory to lipolysis and oxidative phosphorylation.

In an attempt to examine whether adenosine has similar antilipolytic actions in vivo, Shechter (1982) compared the actions of adenosine deaminase in hormonally-stimulated strips of adipose tissue to its actions on stimulated, isolated adipocytes. He found adenosine deaminase to potentiate lipolysis in isolated adipocytes, supporting findings by Turpin et al. (1977). However, adenosine deaminase had no effect on lipolysis in strips of adipose tissue. Shechter (1982) concluded that adenosine has no physiological role in intact adipose tissue. However, another conclusion is possible. Because the strips were not blood-perfused, any agent must diffuse to act at all interstitial sites. Adenosine deaminase is a large protein molecule and may not have diffused to all areas of the strips. Therefore, all endogenous adenosine may not have been degraded, making results from these studies inconclusive.

Other in vivo studies have shown similar results to the in vitro studies. The metabolic actions of adenosine in unstimulated adipose tissue are unclear. In a hormone-stimulated system, adenosine acts to inhibit lipolysis. Infusion of adenosine did not alter the basal glycerol release from canine adipose tissue (Fredholm and Sollevi, 1977; Sollevi and Fredholm, 1981b). In addition, agents that increase the endogenous adenosine concentration did not affect the basal lipolytic rate (Fredholm and Sollevi, 1978; Sollevi and Fredholm, 1981b). These authors concluded that adenosine appears to have no action on the basal lipolytic rate. However, if the basal lipolytic rate is already maximally

inhibited by endogenous concentrations of adenosine (Turpin et al., 1977), infusions of adenosine would not be expected to reduce the basal lipolytic rate further.

The effects of adenosine on stimulated glycerol release in vivo are more certain. Dipyridamole and EHNA (agents that increase the endogenous adenosine concentration) significantly decreased the lipolytic response to nerve stimulation and to the intraarterial administration of norepinephrine (Fredholm and Sollevi, 1977; Sollevi and Fredholm, 1981b; Sollevi et al., 1981). Theophylline, a competitive blocker of adenosine, enhanced the lipolytic effect of nerve stimulation. Theophylline also reversed the antilipolytic effects of dipyridamole and EHNA, indicating that when the endogenous adenosine concentration is increased, lipolysis is inhibited (Sollevi et al., 1981). These metabolic actions of adenosine support the local control hypothesis. As the metabolic rate is increased and adenosine is released, the negative feedback effects of adenosine would blunt the metabolic stimulation. Vasodilation would balance perfusion to the increased metabolic rate and maintain tissue integrity.

In attempts to localize the site of the antilipolytic actions of adenosine, Ebert and Schwabe (1973) prevented the cellular uptake of adenosine by administration of dipyridamole. The inhibition of hormone-stimulated lipolysis by adenosine was enhanced in the presence of dipyridamole, indicating that adenosine was acting at the outer cell surface. The lipolysis induced by dibutyryl cyclic-AMP was not inhibited by adenosine (Ebert and Schwabe, 1973). Dibutyryl cyclic-AMP stimulates lipolysis beyond the level of adenylate cyclase, by activation of the hormone-sensitive lipase. These studies indicate that adenosine acts

extracellularly to inhibit lipolysis, at least in part, by reduction of the levels of cyclic-AMP.

Stimulation of glucose transport

In addition to effects on lipolysis, adenosine has been reported to have insulin-like effects in that adenosine increases glucose uptake in adipose tissue (Dole, 1962). This regulatory action would serve to enhance re-esterification and could limit the accumulation of intracellular free fatty acids. Schwabe et al. (1974) studied the interactions of adenosine and insulin in isolated adipocytes. In concentrated cell suspensions, insulin limited the catecholamine-induced accumulation of cyclic AMP and lipolysis. However, insulin was unable to antagonize the accumulation of cyclic AMP in a dilute cell suspension. Addition of 0.1 μM adenosine to the dilute cell suspension produced a situation similar to that seen with concentrated cell suspensions. Insulin was now able to antagonize the catecholamine effects. Adenosine (0.1 μM) also enhanced the stimulation by insulin of glucose oxidation while adenosine deaminase reduced the action of insulin. The combination of insulin and adenosine produced a greater effect than that seen with either agent alone. These data indicate that adenosine facilitates the actions of insulin to limit lipolysis and to stimulate glucose uptake.

Taylor and Halperin (1979) reported that micromolar concentrations of adenosine alone increased glucose transport in unstimulated, isolated adipocytes. Adenosine further enhanced the maximal transport as increased by insulin, supporting earlier findings of Schwabe et al. (1974). Removal of adenosine by adenosine deaminase decreased the response to insulin (Schwabe et al., 1974; Taylor and Halperin, 1979).

To further examine the interactions of catecholamines, insulin, and adenosine, Souness et al. (1983) incubated adipocytes with adenosine deaminase to remove endogenous adenosine. Addition of 0.1 μM N-6-phenylisopropyladenosine, an adenosine analog that is slowly degraded by adenosine deaminase, increased the rate of re-esterification of free fatty acids from the incubation medium. The effect of insulin alone was similar to that of the adenosine analog. The effects of both agents were enhanced in the presence of glucose. Neither agent alone had much effect on the norepinephrine-induced release of free fatty acids. However, the combination of the two agents strongly inhibited lipolysis. This inhibition was enhanced in the presence of glucose. Adenosine clearly acts to facilitate the action of insulin on glucose uptake in adipose tissue in vitro. No study of the action of adenosine on glucose metabolism in adipose tissue in vivo has been reported.

Evidence exists for a relationship between adenosine and blood flow in adipose tissue, and many studies have indicated a relationship between adenosine and metabolism in vitro. The first detailed study describing the interrelationships of blood flow and metabolism in adipose tissue, possibly linked by adenosine, is that of McCoy (1980). He reported a consistent relationship between tissue metabolic parameters and blood flow, between indices of metabolism and tissue adenosine content, and between tissue adenosine content and blood flow. McCoy's study provided evidence that metabolic regulation of blood flow exists in adipose tissue and may be mediated by adenosine. However, no cause-and-effect relationship between changes in tissue adenosine and vascular events has been demonstrated. The metabolic actions of adenosine pro-

posed *in vitro* have not been clearly demonstrated *in vivo*. Further studies of local control should explore the extent to which adenosine modulates both metabolic and circulatory events in adipose tissue.

RATIONALE

Evidence for a close association between metabolism and blood flow exists in adipose tissue. Lipolysis initiated by the infusion of norepinephrine or other lipolytic hormones is accompanied by vasodilation. The hyperemic response to norepinephrine is of interest, considering that intravenous norepinephrine causes vasoconstriction in most other tissues.

The mechanism by which intravenous norepinephrine causes vasodilation is controversial. One theory proposes that the vascular and metabolic responses are the result of a direct action of norepinephrine on $beta_1$-receptors. The theory also proposes that $beta_1$-receptors at the vasculature in adipose tissue, rather than $beta_2$-receptors as found in other tissues, mediate the vasodilation. When beta-blockade has been used to abolish the vasodilation, the metabolic responses to norepinephrine have been inhibited as well, leaving open the possibility that the two responses are not separate events but are tightly associated. Norepinephrine may, instead, act by an indirect mechanism, related to the release of free fatty acids. The release of water-insoluble fatty acids is dependent on a sufficient supply of albumin. When lipolysis is stimulated, without an increase in the delivery of albumin (vasodilation), free fatty acids accumulate intracellularly and inhibit lipolysis. This metabolic theory is that the vasodilation is "functional" and permits continued lipolysis.

If a signal linked to the level of lipolysis could trigger vasodilation, the rate of blood flow could be matched to the release of free fatty acids. Adipose tissue produces adenosine, a potent vasodilator. Tissue levels of adenosine increase after intravenous norepinephrine and correlate inversely with vascular resistance. However, a cause-and-

effect relationship between adenosine and changes in blood flow has not yet been established.

Adenosine also has metabolic effects that are in keeping with the theory of metabolic regulation of blood flow. Adenosine stimulates glucose transport in vitro. Glucose is a necessary substrate for re-esterification. An increased rate of re-esterification during lipolysis would insure that an overaccumulation of free fatty acids does not occur. Adenosine has also been shown to inhibit the rate of lipolysis induced by catecholamines. By decreasing the rate of lipolysis, adenosine can prevent an overstimulation of the tissue. Through its metabolic and vascular actions, adenosine could modulate both tissue metabolism and circulation to maintain tissue function.

I hypothesize that adenosine has a role in the regulation of blood flow in unstimulated adipose tissues and in tissues in which vasodilation and lipolysis have been induced by norepinephrine. In addition, metabolism and blood flow may be linked through the production of adenosine, and adenosine may regulate adipose tissue metabolism in vivo. If adenosine is the link, removal of its actions should alter the relationship between metabolism and blood flow. An adenosine receptor blocker, theophylline, or an enzyme that specifically degrades adenosine, adenosine deaminase, will be given to alter the vascular and metabolic actions of adenosine. The results from these studies should indicate whether adenosine is a mediator of the vascular and metabolic events in adipose tissue, supporting the concept of metabolic control of blood flow.

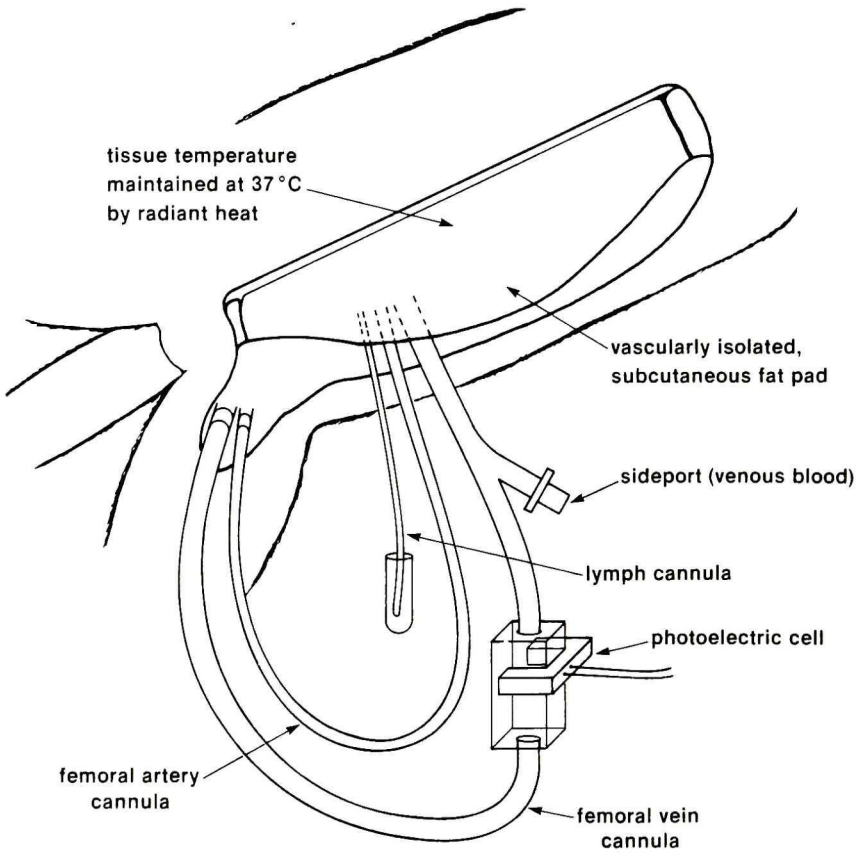
MATERIALS AND METHODS

ANIMAL PREPARATION

Adult, female mongrel dogs were received at the University and determined to be free of microfilaria before assignment to one of the experimental protocols. All animals were subjected to the same surgical procedures. Animals weighing between 13 and 29 kilograms were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and supplements were given as necessary. Animals were intubated and ventilated with room air with a Harvard positive pressure respirator. One carotid artery and both jugular veins were cannulated for withdrawal of arterial samples and infusion of drugs, respectively. Phasic and mean arterial blood pressures were measured by means of a Statham pressure transducer (P23b) and recorded on a Gould recorder (model 2400). Adjustments in minute ventilation and/or administration of sodium bicarbonate intravenously maintained blood gases and pH in the physiologic range (pH 7.40±0.03; PO_2 greater than 80 mmHg; PCO_2 between 35 and 45 mmHg). Animals were positioned on a heating pad, and body temperature was measured rectally. A temperature controller (Yellow Springs Instruments) was connected to the heating pad so that the body temperature could be maintained at 37-38°C.

The subcutaneous, inguinal fat pad on one side was vascularly isolated and denervated according to the method of Rosell (1966) (see Figure 2). A thermocautery was used to separate the adipose tissue from the overlying skin and from the abdominal wall below. The cephalic and caudal ends of the pad were doubly ligated. The isolation procedure left the artery and vein of the fat pad intact. The nerve supplying the fat pad was severed in all experiments. Sodium heparin (1000 U/kg, i.v.) was given prior to cannulation and supplemented every hour

Figure 2. Subcutaneous, inguinal fat pad of dogs *in situ*. The fat pad is vascularly isolated and autoperfused by shunting blood from the femoral artery. Venous outflow is directed through a drop counter for the measurement of blood flow and returned to the femoral vein. Tissue temperature is maintained at 36-37° C. In the adenosine deaminase experiments, a lymph vessel is cannulated and the effluent is collected.


tissue temperature
maintained at 37 °C
by radiant heat
vascularly isolated,
subcutaneous fat pad
sideport (venous blood)
lymph cannula
photoelectric cell
femoral artery
cannula
femoral vein
cannula

(250 U/kg, i.v.). Lipolytic parameters do not change significantly with the administration of heparin (Ballard et al., 1971). The fat pad was autoperfused by shunting blood from the femoral artery, using tubing that permitted close intraarterial injections. The venous outflow was directed through a drop counter (photoelectric cell, Grass model PTTI) for the measurement of blood flow and was returned to the femoral vein. The venous circuit included a side port between the fat pad and the drop counter for the collection of venous blood samples.

In the adenosine deaminase series, all animals received a priming dose of Krebs buffer (pH 7.4; 800 ml/20 kg, i.v.; given in the first hour). The Krebs buffer consisted of 118.0 mM NaCl, 27.4 mM $NaHCO_3$, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 1.0 mM KH_2PO_4. A maintenance infusion of Krebs (150 ml/20 kg, i.v.; each hour) was given over the remainder of the experiment. The Krebs infusion insured that the animals were well-hydrated and has been shown not to affect significantly plasma electrolytes (McKenzie, unpublished observations). The infusion rate was adjusted so that systemic arterial pressure did not increase more than 10 mmHg above pre-experimental values and so that the hematocrit was maintained between 35% and 45%.

In the adenosine deaminase series, several of the lymph vessels draining the fat pad were isolated, and one was selected that permitted cannulation. The remaining vessels were ligated so that the lymph flow was shunted into the cannulated vessel. Cannulation of one vessel allowed the accumulating lymph to pass from any vessel into the collection tube. No ballooning of the ligated vessels was observed, verifying that they were interconnected. Cannulation involved introducing a 20 gauge Angiocath (Deseret) into the lymph vessel. A saline-filled length

of PE-90 tubing was attached to the Angiocath and extended to a collection vessel. The lymph fluid was assayed for adenosine deaminase activity as described below.

EXPERIMENTAL DESIGN

Pre-experimental period

After isolation was complete, the fat pad was covered with saline-soaked gauze and plastic wrap to prevent dehydration. The tissue temperature was maintained at 36-37° C. The tissue temperature and blood flow were allowed to equilibrate for 30 minutes.

After the equilibration period, control hemodynamic parameters were recorded. Arterial and venous (fat pad) blood was sampled (0.5-1.0 ml) anerobically for the determination of pH, PO_2, PCO_2 (ABL, Radiometer) and hematocrit. Arterial and venous blood (5 to 7 ml) was also collected into plastic test tubes packed in ice, centrifuged immediately at 3000 rpm (model J-21B, Beckman Instruments), and the plasma decanted and stored for later analyses of plasma metabolites as described below.

During the experimental period, animals were subjected to one of the protocols in either the theophylline or the adenosine deaminase series. At the end of the experimental period, hemodynamic parameters were recorded and blood was sampled for blood gas and metabolite analyses. The fat pad was then frozen *in situ* by clamping with aluminum tongs pre-cooled to the temperature of liquid nitrogen. Once the tissue was clamped, the section that was not frozen and that was still connected to the animal was immediately severed with a scalpel. The tongs and the frozen tissue were quickly immersed in liquid nitrogen until the tissue was prepared as described below.

Experimental period

Theophylline Series, N=41

Pilot studies (N=5) were done in which theophylline was administered intraarterially to limit its systemic effects. To test the ability of theophylline to block the vascular effects of exogenous adenosine, bolus injections of adenosine were given before and during infusion of theophylline.

In studies with intravenously administered theophylline, 36 animals were randomized into one of four groups. Two groups of animals were used to examine the effects of time or theophylline on unstimulated tissues: saline-saline (control, Sal-Sal; N=9) and theophylline-saline (Th-Sal, N=9). Two groups of animals were used to examine the effects of intravenous norepinephrine alone or in combination with theophylline: saline-norepinephrine (Sal-NE; N=9) and theophylline-norepinephrine (Th-NE; N=9). In all animals, the vascular response to intraarterial boluses of adenosine (10^{-8}, 10^{-7} and 10^{-6} moles) was recorded. When blood flow had returned to the value immediately preceding the administration of the bolus (pre-bolus levels) (0 min), an intravenous infusion of saline (control) or theophylline (10 mg/kg) was given over 13 min. As seen in Figure 3, at 0 min, animals are divided with respect to the presence or absence of theophylline: the saline-treated groups (Sal-Sal and Sal-NE) and the theophylline-treated groups (Th-Sal and Th-NE). At the end of the theophylline infusion (13 min), the low dose (10^{-8} moles) of adenosine was given, and the vascular response was recorded. Arterial and venous blood was sampled when the blood flow had returned to pre-bolus levels (20 min).

At this time (20 min), each major treatment group was subdivided

into two groups with respect to the presence or absence of norepinephrine. One group was designated to receive a second intravenous infusion of norepinephrine, the other saline. There were now 4 groups of 9 animals each. The control group (Sal-Sal) received saline for both infusions and provided information on the effects of time alone on the preparation. The effects of theophylline on unstimulated tissues was examined in the group that received theophylline followed by saline (Th-Sal). The vascular and metabolic effects of intravenous norepinephrine were examined in the group that received saline followed by norepinephrine (Sal-NE). The final group was used to examine the effects of theophylline on norepinephrine-stimulated tissue (Th-NE).

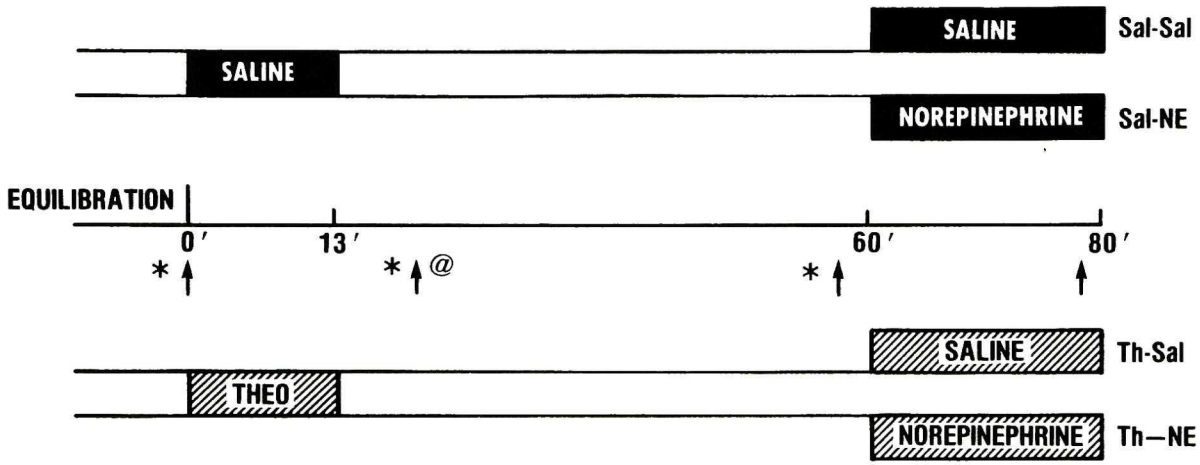
At 20 min, the norepinephrine groups (Sal-NE and Th-NE) received an intravenous bolus of glucose (0.3 ml/kg of 40% glucose) followed by an intravenous infusion of 6% glucose for 20 min to raise plasma levels to 150 mg% in order to elicit a maximal response to norepinephrine (McCoy, 1980). To determine the responses of untreated tissues, and because glucose infusion *per se* has not been shown to alter hemodynamics or metabolism (McCoy, 1980), animals in the unstimulated groups were not treated with glucose.

Hemodynamic events were recorded over the entire procedure. At 50 min, the vascular response to a bolus of 10^{-8} moles adenosine was observed. When blood flow had returned to pre-bolus levels, arterial and venous blood was sampled. At 60 min, the second intravenous infusion of saline or norepinephrine (0.5 µg/kg/minute) was given for 20 min. At 80 min, arterial and venous blood was sampled and the fat pad was freeze-clamped *in situ*.

Blood samples collected from the animals in the theophylline studies were analyzed for plasma free fatty acids, plasma glucose, and

Figure 3. The time course of the experiments with theophylline. An intravenous infusion of saline or theophylline was given (0 min to 13 min) before an intravenous infusion of saline or norepinephrine (60 min to 80 min). Sal-Sal=animals that received saline only. Sal-NE=animals that received saline followed by norepinephrine. Th-Sal=animals that received theophylline followed by saline. Th-NE=animals that received theophylline followed by norepinephrine. The animals that received norepinephrine were given an intravenous infusion of glucose to maintain plasma glucose at 150 mg%. Asterisks indicate the times of administration of an intraarterial bolus of adenosine. Arrows indicate the times of sampling arterial and venous blood to assay for glucose, glycerol and fatty acids.

TIME COURSE OF THE THEOPHYLLINE SERIES



* I.A. BOLUS 10^{-8} MOLES ADENOSINE
↑ PLASMA GLUCOSE, GLYCEROL, FATTY ACIDS
@ PLASMA GLUCOSE 150 mg% IN NOREPINEPHRINE ANIMALS

plasma glycerol. The frozen tissue was assayed for tissue adenosine, AMP deaminase activity, and 5'-nucleotidase activity.

Adenosine Deaminase Series, N=28

In preliminary studies (N=3), lymph was collected during the intraarterial infusion of adenosine deaminase and assayed for the presence of the enzyme. Activity was expressed as μmoles adenosine converted per minute per ml of lymph. The adenosine deaminase activity measured in the lymph was used to determine whether sufficient adenosine deaminase was present to degrade the concentration of adenosine *in vivo*. We have assumed that lymph fluid accurately represents interstitial fluid. For example, in our laboratory, one experiment has indicated that when adenosine deaminase (200 μmoles/min/ml Krebs) was infused intraarterially, the lymph concentration approached 0.07 μmoles/min/ml lymph. This activity was measured under optimal substrate conditions (V_{max}) and does not represent the activity *in vivo*.

To determine whether this activity *in vitro* was sufficient to degrade adenosine at *in vivo* concentrations, the following estimations were made. Sollevi and Fredholm (1981a) reported a maximal tissue adenosine content of 2.8 nmoles/g after sympathetic nerve stimulation. In adipose tissue, the interstitial fluid is approximately 10% of the tissue weight (Linde and Chisholm, 1975), and the average tissue weighs 50 grams (Sollevi and Fredholm 1981a, Sollevi and Fredholm 1981b). Therefore, in a 50 gram tissue, 2.8 nmoles/g is equivalent to a total of 140 nmoles. The total interstitial volume is 5 ml. If one assumes the entire tissue content of adenosine to be extracellular, the interstitial concentration of adenosine is 140 nmoles/5 ml or 28 μM. The total adenosine deaminase activity resulting from infusion was 0.35

μmoles/min (0.07 μmoles/min/ml x 5 ml). The activity *in vivo* of the adenosine deaminase at this substrate concentration was determined with the Michaelis-Menten velocity equation**:

$$V_{(\text{in vivo})} = \frac{V_{max} \times S_{(\text{in vivo})}}{K_m + S_{(\text{in vivo})}}$$

$$V = \frac{0.35\ U \times 28\ \mu M}{40\ \mu M + 28\ \mu M}$$

$$V = \frac{0.35\ U \times 28}{68}$$

$$V = 0.144\ U$$

(144 nmoles of adenosine can be degraded in one minute)

** V_{max}= velocity at saturating concentrations of substrate; maximal velocity
$V_{\text{in vivo}}$= velocity at the estimated concentration of adenosine *in vivo*
$S_{\text{in vivo}}$= estimated concentration of substrate (adenosine) *in vivo*
K_m= substrate concentration at which the adenosine deaminase works at half-maximal velocity (Arch and Newsholme, 1978)

The total tissue content of adenosine was estimated at 140 nmoles. Therefore, 0.07 μmoles/min/ml lymph of adenosine deaminase can degrade the entire tissue content of adenosine in less than one minute. Moreover, this activity would continue to effectively degrade any adenosine released at a rate of less than 144 nmoles/minute. However, a release rate of this magnitude would produce interstitial concentrations of approximately 30 μM. Fredholm and Sollevi (1981) have estimated a basal adenosine release rate of 0.1 nmole/min/g. The rate increases with sympathetic stimulation to as much as 1.2 nmole/min/g. The levels of adenosine deaminase that were achieved in our pilot study would, at steady state in unstimulated tissue, maintain an interstitial adenosine concentration of 0.1 μM.

Adenosine deaminase (Sigma Type I, Sigma Chemical Co.) was

diluted in Krebs buffer (pH 7.4-7.9, 200 μmoles/min/ml) and dialyzed overnight against several changes of buffer. The dialysis tubing (Fisher Products and A.H. Thomas, Co.) allows the passage of compounds of molecular weight less than 12,000. The activity of the enzyme was tested after dialysis and averaged 130 μmoles/min/ml Krebs.

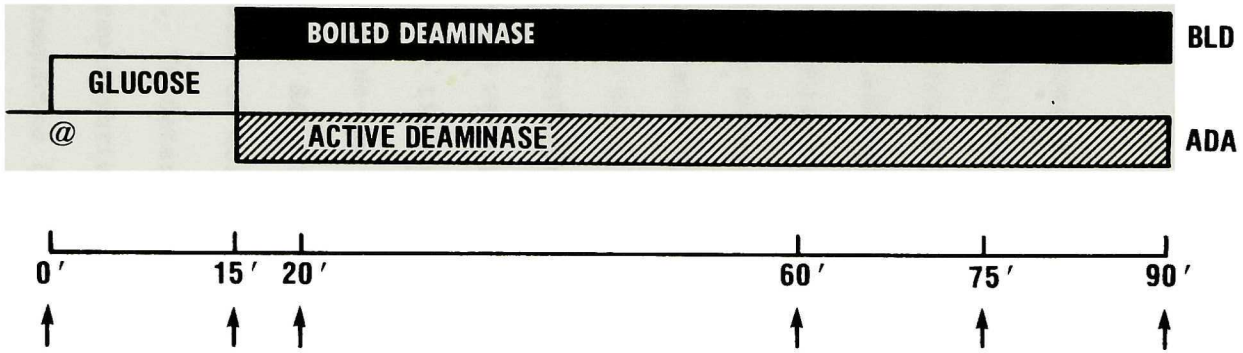
In the adenosine deaminase series, one group of animals (ADA; N=7) was used to determine the effects of intraarterial infusion (0.05 to 0.1 ml/min) of adenosine deaminase on basal, unstimulated tissue. To provide control data for the effects of infusion alone and the effects of time on the preparation, a second group of animals (BLD; N=6) received a heat-inactivated (boiled) adenosine deaminase preparation.

In all animals, cannulation of lymph vessels was attempted but the collection of lymph samples was not always successful. In 50% of the animals, a cannula was placed but the lymph flow was extremely low or stopped at some time into the experimental procedure. Whenever the collection was successful, lymph was collected before and at several points during the adenosine deaminase infusion. The lymph was refrigerated for later analysis of adenosine deaminase activity.

After equilibration, arterial and venous blood and lymph were sampled (0 min; Figure 4). An intravenous bolus and infusion of glucose was given to maintain plasma glucose at approximately 150 mg%. At 15 min, an intraarterial infusion (0.05-0.1 ml/min) of boiled adenosine deaminase (control, BLD) or active adenosine deaminase (130 μmoles/min/ml, ADA) was begun and continued throughout the remainder of the experiment. Pilot studies indicated that the infusion of active adenosine deaminase caused an initial vasodilation that lasted 5 to 6 min. At 20 min, arterial and venous blood was sampled to determine whether a change in

Figure 4. The time course of experiments with adenosine deaminase in basal, unstimulated tissues. All animals received an intravenous infusion of glucose (0 min to 90 min) to maintain plasma glucose at 150 mg%. An intraarterial infusion of boiled deaminase (control, BLD) or active adenosine deaminase (130 U/ml, ADA) was given from 15 min to 90 min. Arrows indicate the times of sampling arterial and venous blood to assay for glucose and glycerol.

TIME COURSE OF THE DEAMINASE SERIES: BASAL TISSUES



↑ PLASMA GLUCOSE, GLYCEROL

@ PLASMA GLUCOSE AT 150 mg%

in metabolism could be associated with the transient vasodilation. At 60 min, arterial and venous blood were sampled and hemodynamic parameters recorded. A second intravenous infusion (0.1 to 0.2 ml/min) of saline was then begun and continued for 30 minutes. At 90 minutes, blood was sampled, hemodynamic parameters were recorded, and the tissue was freeze-clamped in situ.

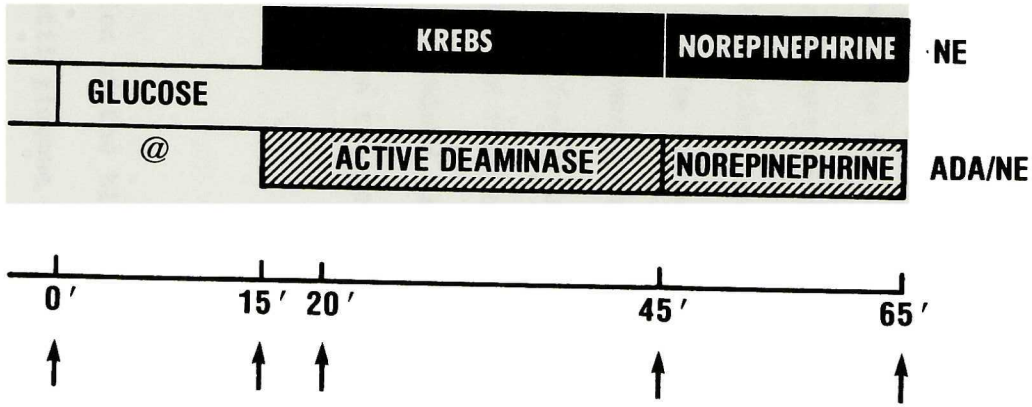
The time of the protocol used for the studies in unstimulated tissues (groups BLD and ADA) was chosen to allow for sufficient amounts of adenosine deaminase to accumulate in the interstitial space and to degrade the extracellular adenosine. The purpose of the second, intravenous infusion was to control for the effects of infusion of a lipolytic agent to be given in subsequent studies. However, the studies in unstimulated tissues indicated that the indices of the hemodynamic and metabolic states fell over time. This decline may be related to a general deterioration of the preparations. To insure that the studies of hormone-stimulated metabolism were performed in tissues that were capable of a maximal response, the time course of the protocol for the groups that received norepinephrine was shortened by approximately 30 min.

To provide control data for the vascular and metabolic effects of an intravenous infusion of norepinephrine, six animals received norepinephrine alone (NE). To determine the effects of adenosine deaminase on the responses to norepinephrine, six animals received adenosine deaminase followed by norepinephrine (ADA/NE).

The protocol for the studies of stimulated tissues is shown in figure 5. After equilibration (0 min), arterial and venous blood and lymph were sampled, and control hemodynamic measurements were taken. An intravenous bolus and infusion of glucose was given as described for the

Figure 5. The time course of experiments with adenosine deaminase in stimulated tissues. All animals received an intravenous infusion of glucose (0 min to 65 min) to maintain plasma glucose at 150 mg%. An intraarterial infusion of Krebs (control, NE) or active adenosine deaminase (130 U/ml, ADA/NE) was given from 15 min to 65 min. All animals received an intravenous infusion of norepinephrine from 45 min to 65 min. Arrows indicate the times of sampling arterial and venous blood to assay for glucose and glycerol.

TIME COURSE OF THE DEAMINASE SERIES: STIMULATED TISSUES



↑ PLASMA GLUCOSE, GLYCEROL

@ PLASMA GLUCOSE AT 150 mg%

studies in unstimulated tissues. At 15 min, an intraarterial infusion (0.05 to 0.1 ml/min) of Krebs buffer (NE) or adenosine deaminase (130 μmoles/min/ml, ADA/NE) was begun and continued throughout the remainder of the experiment. At 20 min, during the initial vasodilation, blood was sampled. At 45 min, hemodynamic parameters were recorded and blood and lymph were sampled. An intravenous infusion (0.6 ml/min) of norepinephrine (0.25 μg/kg/min) was begun and continued for 20 min. This dose of norepinephrine was lower than that used in the theophylline studies. If a maximal stimulatory concentration were used, adenosine might not be capable of antilipolytic action. Therefore, the administration of adenosine deaminase would not be expected to alter the lipolytic response. At 65 min, hemodynamic measurements were made, blood and lymph were sampled, and the tissues were freeze-clamped in situ.

Blood samples collected from the animals in the adenosine deaminase series were analyzed for blood gases, hematocrit, plasma glycerol, and plasma glucose. The frozen tissues were assayed for tissue adenosine content.

CHEMICAL ANALYSES

Plasma metabolites

After centrifugation of the blood samples, an aliquot of the plasma was refrigerated until glucose measurements were made. The remainder of the plasma was frozen for glycerol and fatty acid measurements. These measurements were performed within one week.

Plasma glucose

Plasma glucose was determined by the glucose oxidase method using a Beckman II glucose analyzer. When plasma is mixed with oxygen in the presence of glucose oxidase, the rate of oxygen depletion is directly

proportional to the amount of glucose present and is displayed as mg%. Glucose measurements are reproducible within 3 mg% and are converted to μmoles/ml plasma.

Plasma free fatty acids

Plasma free fatty acids were determined colorimetrically using the copper binding method of Itaya and Ui (1965). Plasma was mixed with chloroform, and the mixture was centrifuged. The free fatty acids were extracted into the chloroform phase. The aqueous layer was removed by aspiration. The chloroform-fatty acid mixture was then mixed with a copper nitrate solution. The copper binds to the free fatty acids in a ratio of one copper molecule:fatty acid. After centrifugation, the excess copper nitrate which remains in the aqueous phase was removed by aspiration. A sodium diethyldithiocarbamate solution was then added to the chloroform-fatty acid mixture to develop a yellow color upon reaction with the bound copper. The samples were read in a spectrophotometer at 440 nm. The absorbance of the samples was compared to that of palmitate standards. Plasma free fatty acids were measured in μmoles/ml plasma. Detection of free fatty acids was possible at levels greater than 0.001 μmoles/ml plasma, and the assay was reproducible within 15%.

Plasma glycerol

Plasma glycerol was determined enzymatically by the conversion of NAD^+ to NADH according to the method of Wieland (1965), adapted for the fluorometer. Plasma proteins were precipitated with perchloric acid, and the mixture was centrifuged. Potassium carbonate was added to the supernatants to neutralize the extracts. After a second centri-

fugation, the supernatants were added to a buffer containing hydrazine, cysteine, ATP, glycerol phosphate dehydrogenase, and NAD+. The baseline fluorescence of the buffered samples was recorded at 460 nm. Glycerol kinase was then added to the samples, and the samples were incubated for 2 hours. The glycerol kinase converts glycerol to glycerol 3-phosphate, which is then converted to glyceraldehyde by the action of the dehydrogenase. Concommitantly, NADH is produced. Reduced NAD fluoresces at 460 nm; after the timed incubation, the amount of fluorescence is recorded, the baseline fluorescence is subtracted, and the adjusted fluorescence is compared with that of the glycerol standards. Plasma glycerol was measured in μmoles/ml of plasma. This assay could detect values above 0.001 μmoles/ml plasma, and reproducibility was within an average of 15%. At low plasma glycerol concentrations, reproducibility was often as poor as 30%, as a small absolute difference in fluorometer units was a significant proportion of the total amount measurable.

Tissue metabolites

The frozen adipose tissue was weighed. Sections of the tissue that were appropriately clamped were then stored in liquid nitrogen until they were prepared for metabolite analysis.

Tissue adenosine content

The frozen sections of tissue were placed into a stainless steel mortar and pestle that had been cooled to the temperature of liquid nitrogen. The tissue was pulverized, and an aliquot (approximately 2 g) was placed into a glass test tube that had been cooled in liquid nitrogen. The tube and aliquot were rapidly weighed, the tissue transferred to a plastic tube packed in dry ice, and the glass tube was reweighed.

The difference between the weight of the combined glass tube and tissue and the weight of the tube alone was taken as tissue weight.

To denature the tissue proteins, perchloric acid (1.0 N, 5 ml/gm of tissue) was added. The mixture was homogenized (Polytron) for 2 to 3 min while the plastic tube was packed in ice. The homogenized tissues were centrifuged at 12,000 rpm for 20 minutes. To remove tissue lipids that might interfere with the assay (McCoy, 1980), the supernatants were filtered through glass wool. The filtered extracts were neutralized to pH 7.5-8.0 by the addition of potassium hydroxide. The neutralized extracts were centrifuged at 12,000 rpm to remove the precipitated perchlorate salts. To improve the sensitivity of the assay, concentration of the samples was necessary. The neutralized samples were dried in a water bath (40°C) and resuspended by the addition of 3 ml of a 50 mM NaH_2PO_4/10 mM EDTA buffer, pH 7.4.

Tissue contents of adenosine were determined in the neutralized, acid-precipitated extracts by the enzymatic conversion to uric acid (Olsson, 1970). Briefly, the tissue extract was incubated first with xanthine oxidase, then with nucleoside phosphorylase, and lastly with adenosine deaminase (Boehringer Mannheim). The pools of hypoxanthine, inosine, and lastly adenosine were converted stepwise to uric acid. The tissue contents were measured by the change in absorbance at 292 nm with a dual-beam, dual-wavelength spectrophotometer (Perkin-Elmer model 556). Tissue adenosine content is expressed as nmoles/g tissue. Samples were assayed in duplicate. Metabolite contents as low as 0.020 nmoles/g could be detected and reproducibility was approximately 10%.

Interstitial adenosine deaminase activity

A 25 µl aliquot of each lymph sample was added to a cuvette containing saturating concentrations (3 mM) of adenosine. The linear change in absorbance at 285 nm was recorded as adenosine was converted to inosine. Adenosine deaminase activity (V_{max}) was expressed as nmoles of adenosine deaminated/min/ml lymph.

Tissue AMP deaminase activity

Tissues (approximately 1 g) were homogenized in a potassium phosphate buffer containing mercaptoethanol. The homogenates were incubated in the presence of 4 mM AMP and 0.1 M succinate. The activities of AMP deaminase were determined by the linear change in absorbance at 285 nm as AMP was converted to IMP (Smiley et al., 1967). The activities of AMP deaminase are expressed as µmoles AMP converted/min/g tissue. The minimal activity that could be detected was 10 µmoles/min/g.

Tissue 5'-nucleotidase activity

5'-Nucleotidase activity was determined by the method of Baer et al., (1966). The tissues were homogenized in acetone, vacuum-dried, and rehomogenized in a sodium phosphate buffer (50 mM, pH 7.4). The homogenates were dialyzed overnight against several changes of buffer. The dialyzed extracts were solubilized in sodium deoxycholate, and an aliquot was incubated in AMP substrate (1.0 mM). Tissue aliquots were incubated for 30 and 60 minutes, in the presence and absence of AOPCP, a 5'-nucleotidase inhibitor (Burger and Lowenstein, 1970). The two time points allowed for duplicate samples and insured linearity of the enzymatic reaction. The reactions were terminated by the addition of perchloric acid (1.0 N). The total production of adenosine, inosine, and hypoxanthine was measured as described for the tissue content of

adenosine. In the presence of AOPCP, total production represents non-specific phosphatase activity. Specific 5'-nucleotidase activity was calculated as the total production (in the absence of AOPCP) minus the non-specific production (in the presence of AOPCP). Activity is expressed as nmoles AMP converted/min/g tissue.

CALCULATIONS

Adipose tissue blood flow (ml/min/100g)

$$\text{Absolute blood flow (ml/min)} \times \frac{100}{\text{fat pad weight, g}}$$

Adipose tissue plasma flow (ml/min/100g)

Adipose tissue blood flow (ml/min/100g) x (1 - hematocrit)

Metabolite uptake or release

Glucose Uptake (μmoles/min/100g)

$$([\text{Glucose}]_{\text{arterial}} - [\text{Glucose}]_{\text{venous}}) \times \text{plasma flow}$$

Fatty Acid Release (μmoles/min/100g)

$$([\text{Fatty Acid}]_{\text{venous}} - [\text{Fatty Acid}]_{\text{arterial}}) \times \text{plasma flow}$$

Glycerol Release (μmole/min/100g)

$$([\text{Glycerol}]_{\text{venous}} - [\text{Glycerol}]_{\text{arterial}}) \times \text{plasma flow}$$

Vascular effects of adenosine

Percent increase above basal blood flow

$$\frac{\text{Peak blood flow after the bolus} - \text{blood flow at the beginning of the experiment (0 min)}}{\text{Blood flow at the beginning of the experiment}} \times 100$$

Percent increase above pre-bolus blood flow

$$\frac{\text{Peak blood flow after the bolus} - \text{blood flow immediately preceding the bolus (pre-bolus)}}{\text{Blood flow immediately preceding the bolus (pre-bolus)}} \times 100$$

Absolute change in blood flow

Peak blood flow after the bolus - blood flow immediately preceding the bolus (pre-bolus)

DATA ANALYSIS

Differences among groups were tested for by analysis of variance with repeated measures when appropriate. Significant differences among pairs of means were tested with Duncan's Multiple Range Test at the P=0.05 level. Linear regression analysis was used to test for a linear relationship between variables.

RESULTS

THEOPHYLLINE STUDIES

Hemodynamics

At the onset of the procedure, mean arterial blood pressures and blood flows were similar among groups (Table I). Blood flow through the tissues was stable once the temperature of the tissues had reached 37°C. Blood flows at the onset of the experiment ranged from 2.2 to 14.0 ml/min/100 g. Blood pressures were stable in the saline (Sal-Sal) and theophylline (Th-Sal) control groups over the 80 min experimental procedure. The intravenous infusion of norepinephrine (0.5 µg/kg/min) elicited an immediate increase in mean arterial blood pressure and a reflex decrease in heart rate in both the saline-treated (Sal-NE) and the theophylline-treated (Th-NE) groups. However, by 10 min of norepinephrine infusion, mean pressures were not significantly different from the pre-norepinephrine blood pressures. Norepinephrine increased hematocrit and slightly lowered arterial pH. The animals were well-ventilated, as indicated by the blood gas values over the procedure. Fat pad weights ranged from 17 to 79 g, averaging 39±4 g for the series.

Ability of theophylline to antagonize exogenous adenosine

To limit systemic effects of theophylline, the drug was first given by intraarterial infusion. To test the effectiveness of intraarterial theophylline as an adenosine antagonist, five animals were subjected to these pilot experiments. The vascular response to increasing doses of adenosine was tested. Close, intraarterial boluses (0.1 ml of 0.1, 1, and 10 x 10^{-3} M; 10^{-8}, 10^{-7}, and 10^{-6} moles, respectively) were given. Theophylline (10 mM) was infused intraarterially in a stepwise fashion. After 10 minutes at each rate, the response to the low dose (10^{-8} moles) of adenosine was tested. Because theophylline is a com-

Table I. Initial and final hemodynamic measurements in the theophylline groups.

Initial measurements (0 min)

Group	MABP (mmHg)	Q (ml/min/ 100g)	VR (mmHg·min· 100g)/ml	pH	PO_2 (mmHg)	PCO_2 (mmHg)	Hct (%)
Sal-Sal	137 ±5	7.4 ±0.4	19.0 ±1.4	7.38 ±0.01	89 ±2	38 ±1	39 ±1
Th-Sal	131 ±1	8.4 ±1.0	17.3 ±1.9	7.38 ±0.06	92 ±2	35 ±1	39 ±1
Sal-NE	131 ±5	5.8 ±0.9@	25.3 ±3.3	7.39 ±0.01	90 ±1	33 ±1	42 ±1
Th-NE	132 ±5	7.3 ±0.7	19.2 ±1.6	7.39 ±0.01	89 ±1	33 ±1	40 ±1

Final measurements (80 min)

Group	MABP (mmHg)	Q (ml/min/ 100g)	VR (mmHg·min· 100g)/ml	pH	PO_2 (mmHg)	PCO_2 (mmHg)	Hct (%)
Sal-Sal	132 ±9	4.3 ±0.6	33.7 ±3.2	7.38 ±0.01	85 ±3	35 ±2	42 ±2
Th-Sal	131 ±3	4.5 ±0.5	34.1 ±3.1	7.38 ±0.01	91 ±2	35 ±1	41 ±2
Sal-NE	146 ±5	5.4 ±0.7	26.5 ±2.3	7.33 ±0.02*	80 ±3	35 ±1	52 ±2*
Th-NE	136 ±6	8.1 ±1.4*@+	22.1 ±4.4*@	7.33 ±0.02*	80 ±4	34 ±1	50 ±3*

Values are means±SEM.
MABP=mean arterial blood pressure
Q=blood flow per 100g tissue
VR=vascular resistance
pH, PO_2, PCO_2=arterial blood pH and gases
Hct=hematocrit

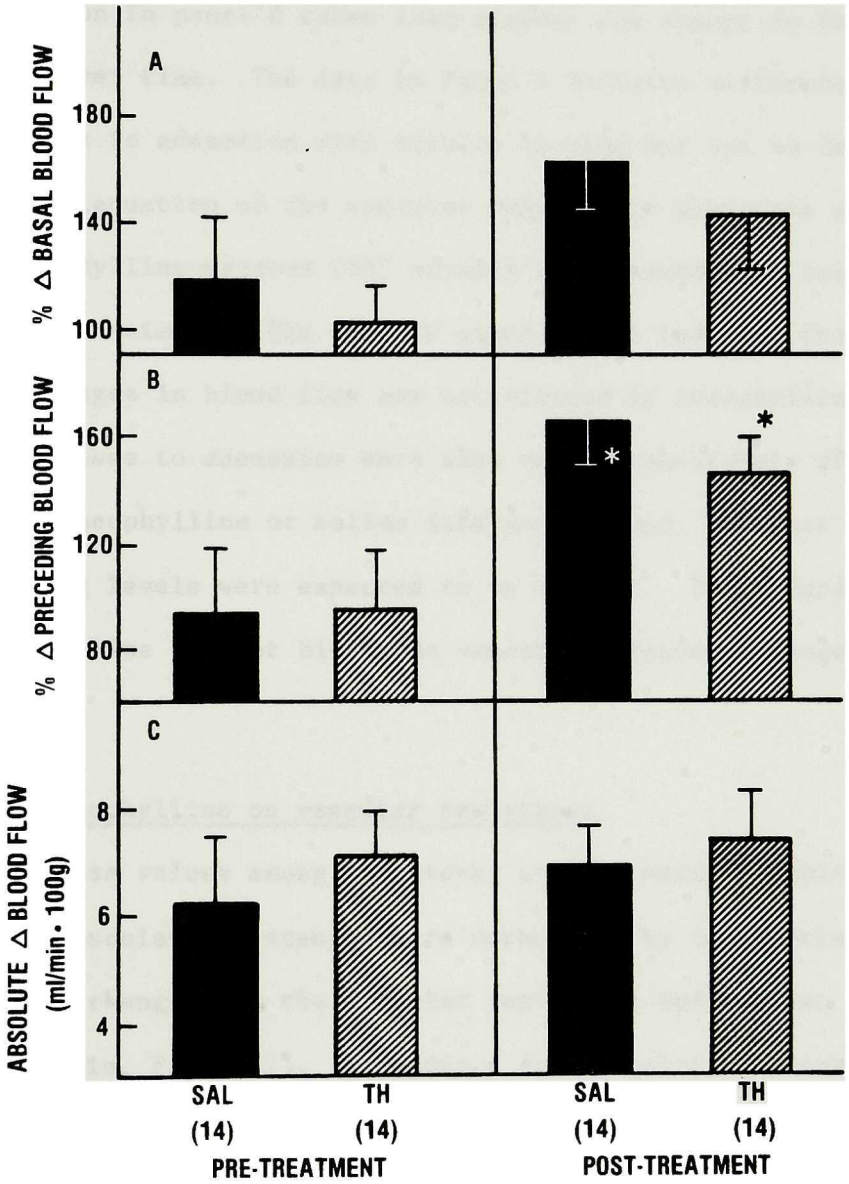
Sal-Sal=saline control
Th-Sal=theophylline control
Sal-NE=norepinephrine
Th-NE=theophylline/ norepinephrine

*=significantly different from Sal-Sal at that time, $P<0.05$.
@=significantly different from Th-Sal at that time, $P<0.05$.
+=significantly different from Sal-NE at that time, $P<0.05$.

petitive blocker, the low dose of adenosine was given so that any blockade by theophylline would not be overcome. No blunting of the vascular effects of adenosine was observed. At a calculated arterial concentration of 10^{-4} M, theophylline caused vasodilation. The infusion was decreased to give an arterial concentration of 10^{-5} M, the maximal theophylline concentration that did not cause vasodilation. After 20 min at this infusion rate, the low dose of adenosine was given. No blockade of the vascular response of adenosine was observed. Therefore, the remaining experiments were designed to infuse theophylline intravenously. Sollevi and Fredholm (1981) reported that theophylline administered intravenously (7.5-10 mg/kg, over 13 min) was able to block the vascular effects of adenosine.

The effects of theophylline (10 mg/kg, i.v.) on the vascular response to an intraarterial bolus of 10^{-8} moles of adenosine are shown in Figure 6. Up to the onset of norepinephrine, all saline animals (Sal-Sal, Sal-NE) were treated similarly, as were all theophylline animals (Th-Sal, Th-NE). Therefore, the responses to adenosine are pooled by class of treatment, saline (SAL) or theophylline (TH). The pre-treatment (left) panels show the responses to 10^{-8} moles of adenosine given immediately before the saline or theophylline infusions (0 min). The post-treatment (right) panels show the responses to boluses that were given immediately before the intravenous infusion of norepinephrine, 60 min after the onset of saline or theophylline administration. Three separate representations of the blood flow response to adenosine are shown in panels A, B, and C. Panel A shows the response calculated as a percent increase in blood flow above the blood flow before drug administration (basal blood flow, 0 min). As seen in panel A, no blockade by theophyl-

Figure 6. Vascular effects of an intraarterial bolus of 10^{-8} moles of adenosine given to saline-treated (SAL) or theophylline-treated (TH) animals. Effects are measured as a percent change (%Δ) from basal flow (0 min) (panel A), as a percent change (%Δ) from immediately preceding flow (panel B), or as the absolute change from immediately preceding blood flow (panel C). The left panels illustrate the vascular responses to adenosine before the administration of saline or theophylline (Pre-treatment). The right panels illustrate the responses to adenosine at 60 min after saline or theophylline, immediately before norepinephrine (Post-treatment). ( )=number of animals. *=significantly different from SAL response pre-treatment, $P<0.05$.

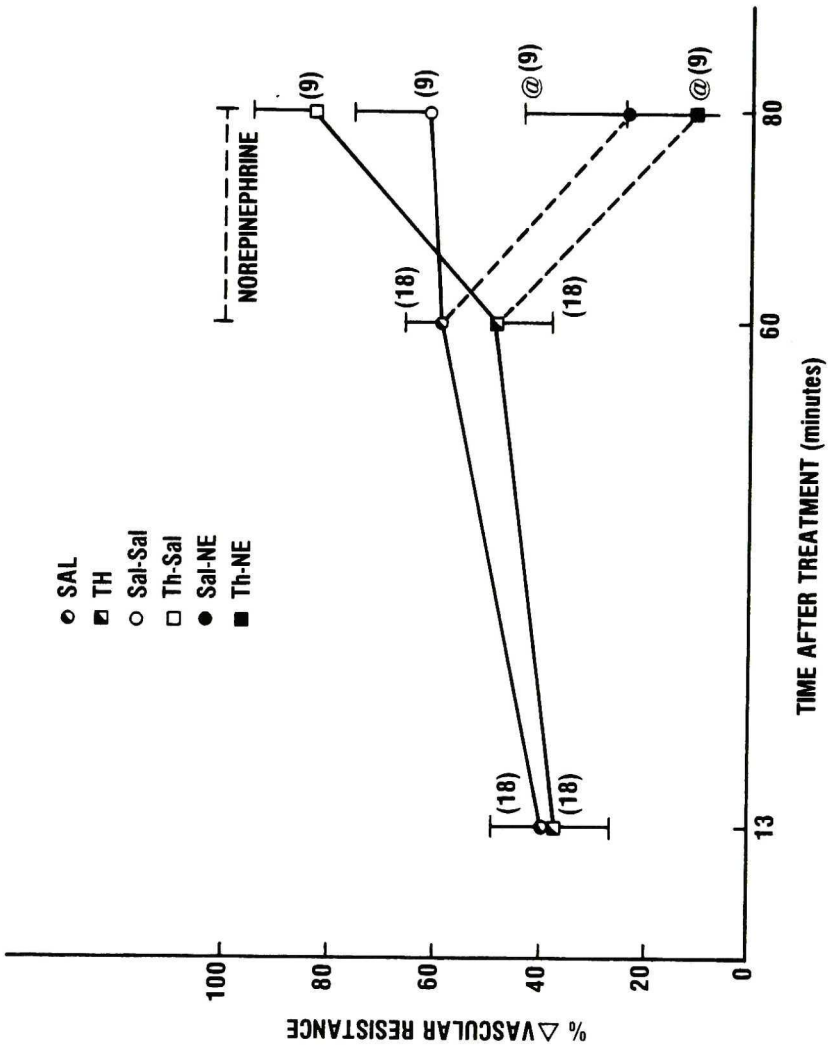

A
B
C
% Δ BASAL BLOOD FLOW
% Δ PRECEDING BLOOD FLOW
ABSOLUTE Δ BLOOD FLOW
(ml/min • 100g)
180
140
100
160
120
80
8
6
4
*
*
SAL
(14)
TH
(14)
SAL
(14)
TH
(14)
PRE-TREATMENT
POST-TREATMENT

line of the responses to adenosine was observed. When the adenosine response is calculated as the percent increase in blood flow above the blood flow that immediately preceded the bolus (panel B), the absolute percentages differ as compared to those in panel A. The method of calculation in panel B takes into account the change in baseline blood flow over time. The data in Panel B indicate differences in the responses to adenosine with respect to time but not to drug treatment. No attenuation of the vascular response to adenosine was observed in theophylline-treated (TH) animals as compared with saline-treated (SAL) animals. The data in panel C also indicate that the absolute changes in blood flow are not altered by theophylline treatment. Responses to adenosine were also tested immediately after completion of theophylline or saline infusion (13 min, data not shown), when the drug levels were expected to be highest. These data indicate that theophylline did not block the vascular effects of exogenous adenosine.

Effect of theophylline on vascular resistance

Because values among individual animals were variable even at 0 min, the vascular resistances were normalized by considering the data as a percent change from the vascular resistance before drug administration (0 min, Figure 7). A tendency for vascular resistance to increase was observed in both saline- and theophylline-treated animals up to the time of norepinephrine infusion (60 min). Theophylline treatment did not elicit an additional increase in baseline vascular resistance as compared to saline, as would be expected if theophylline had blocked the vascular effects of endogenous adenosine. In fact, the percent increases in vascular resistance tended to be lower in theophylline-

Figure 7. Percent change (%Δ) in vascular resistance from the onset of an intravenous infusion of saline or theophylline. The dashed lines indicate the time of intravenous norepinephrine. Values are means±SEM. ( )=number of animals. SAL=all animals that received saline for the first infusion. TH=all animals that received theophylline for the first infusion. Sal-Sal=animals that received saline only. Th-Sal=animals that received theophylline and saline. Sal-NE=animals that received saline and norepinephrine. TH-NE=animals that received theophylline and norepinephrine. @=significantly different from Th-Sal, $P<0.05$.


SAL
TH
Sal-Sal
Th-Sal
Sal-NE
Th-NE
NOREPINEPHRINE
(9)
(9)
@(9)
@(9)
(18)
(18)
(18)
(18)
100
80
60
40
20
0
13
60
80
% ∆VASCULAR RESISTANCE
TIME AFTER TREATMENT (minutes)

treated groups than in saline-treated groups.

Effect of theophylline on the vascular response to norepinephrine

Theophylline did not block the vasodilation that occurred in response to intravenous norepinephrine (0.5 μg/kg/min; Figure 8). As illustrated in Figure 8, vascular resistance decreased significantly over the time of norepinephrine infusion in the groups that received norepinephrine, whether the animal was treated with theophylline or saline. Over a similar time period, vascular resistance tended to increase in the unstimulated groups (Sal-Sal and Th-Sal). Although the increase in the theophylline group (Th-Sal) tended to be greater over the period of norepinephrine infusion than that expected with time alone (Sal-Sal), the difference was not statistically significant.

Effect of theophylline on unstimulated metabolism

All four groups were similar with respect to glucose uptake, glycerol release, and fatty acid release at 0 min (Table II). Metabolic responses between classes immediately after the drug infusion (13 min) and immediately before the onset of norepinephrine (60 min) were normalized by expressing the responses as percent changes from initial values (0 min, Figure 9). Panel A shows the percent changes in glucose uptake in the two classes at peak drug levels and immediately before the infusion of norepinephrine. No significant change in glucose uptake with time was observed. Moreover, theophylline treatment did not cause a decrease in glucose uptake. Panel B shows that glycerol release in both saline- and theophylline-treated animals did not change with time. Thus, there is no evidence that treatment with theophylline stimulated glycerol release. Panel C shows the changes in fatty acid release

Figure 8. Percent change (%Δ) in vascular resistance over the time (60 min to 80 min) of an intravenous infusion of saline (-Sal) or norepinephrine (-NE). Values are means±SEM. ( )=number of animals. Sal-Sal=animals that received saline only. Th-Sal=animals that received theophylline and saline. Sal-NE= animals that received saline and norepinephrine. TH-NE=animals that received theophylline and norepinephrine. *=significantly different from Sal-Sal, $P<0.05$. @=significantly different from Th-Sal, $P<0.05$.

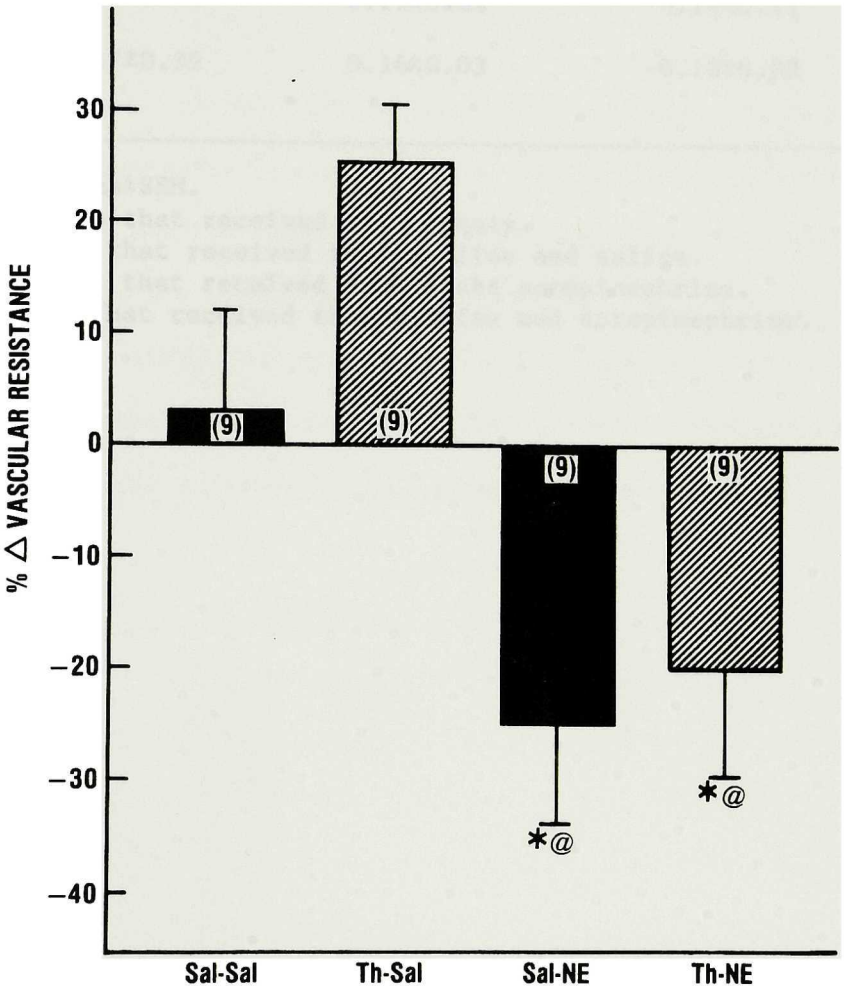
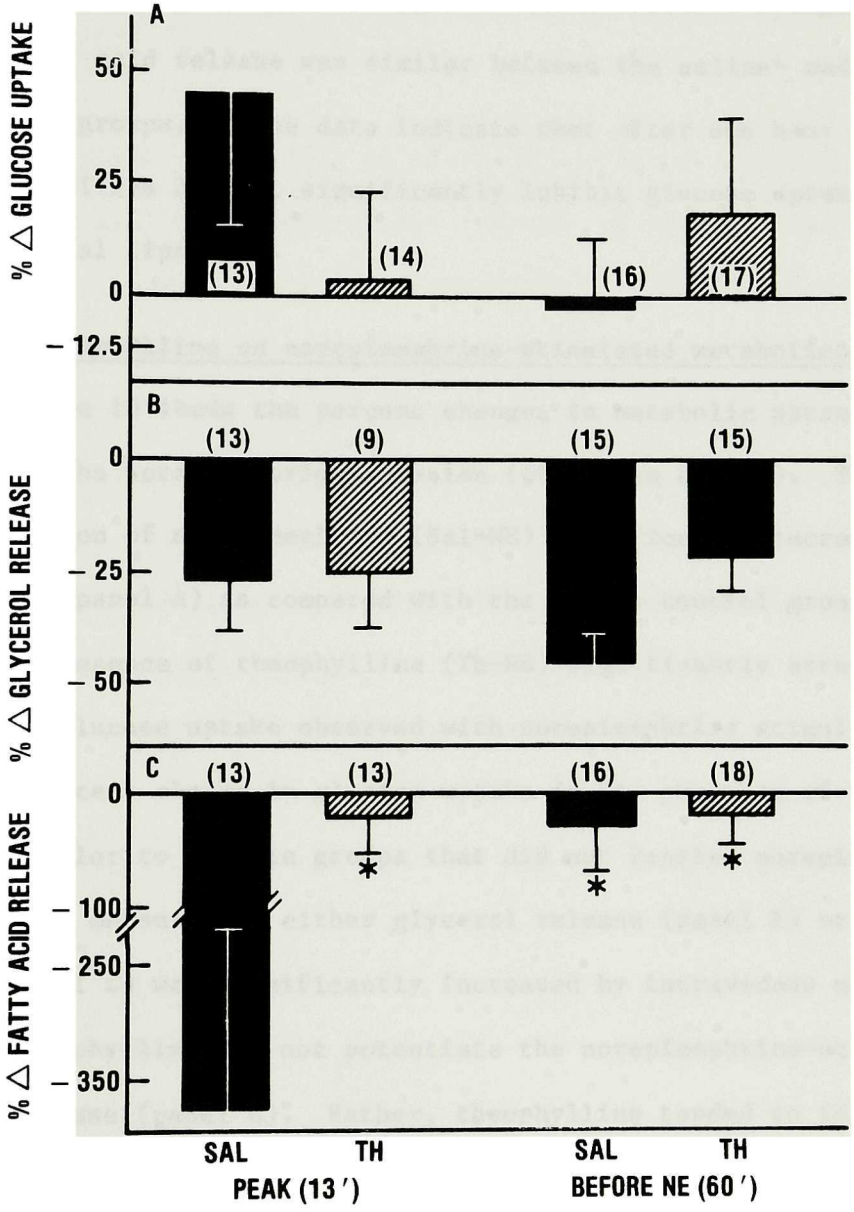

% Δ VASCULAR RESISTANCE
30
20
10
0
−10
−20
−30
−40
(9)
(9)
(9)
(9)
*@
*@
Sal-Sal
Th-Sal
Sal-NE
Th-NE

Table II. Initial metabolic measurements in the theophylline groups.

Group	Glucose Uptake (µmole/min/100g)	Glycerol Release (µmole/min/100g)	Fatty Acid Release (µmole/min/100g)
Sal-Sal	1.86±0.23	0.19±0.04	-0.26±0.13
Th-Sal	2.06±0.31	0.24±0.03	-0.08±0.08
Sal-NE	1.95±0.41	0.11±0.02	0.14±0.11
Th-NE	2.17±0.39	0.16±0.03	-0.18±0.08

Values are means±SEM.
Sal-Sal=animals that received saline only.
Th-Sal=animals that received theophylline and saline.
Sal-NE= animals that received saline and norepinephrine.
TH-NE=animals that received theophylline and norepinephrine.

Figure 9. Percent change (%Δ) in metabolic parameters from the onset of an intravenous infusion of saline or theophylline, measured at peak drug levels (13 min) or immediately before norepinephrine (Before NE, 60 min). Values are means±SEM. ( )= number of animals. SAL=all animals that received saline for the first infusion. TH=all animals that received theophylline for the first infusion. *=significantly different from the response in SAL animals at Peak, $P<0.05$.

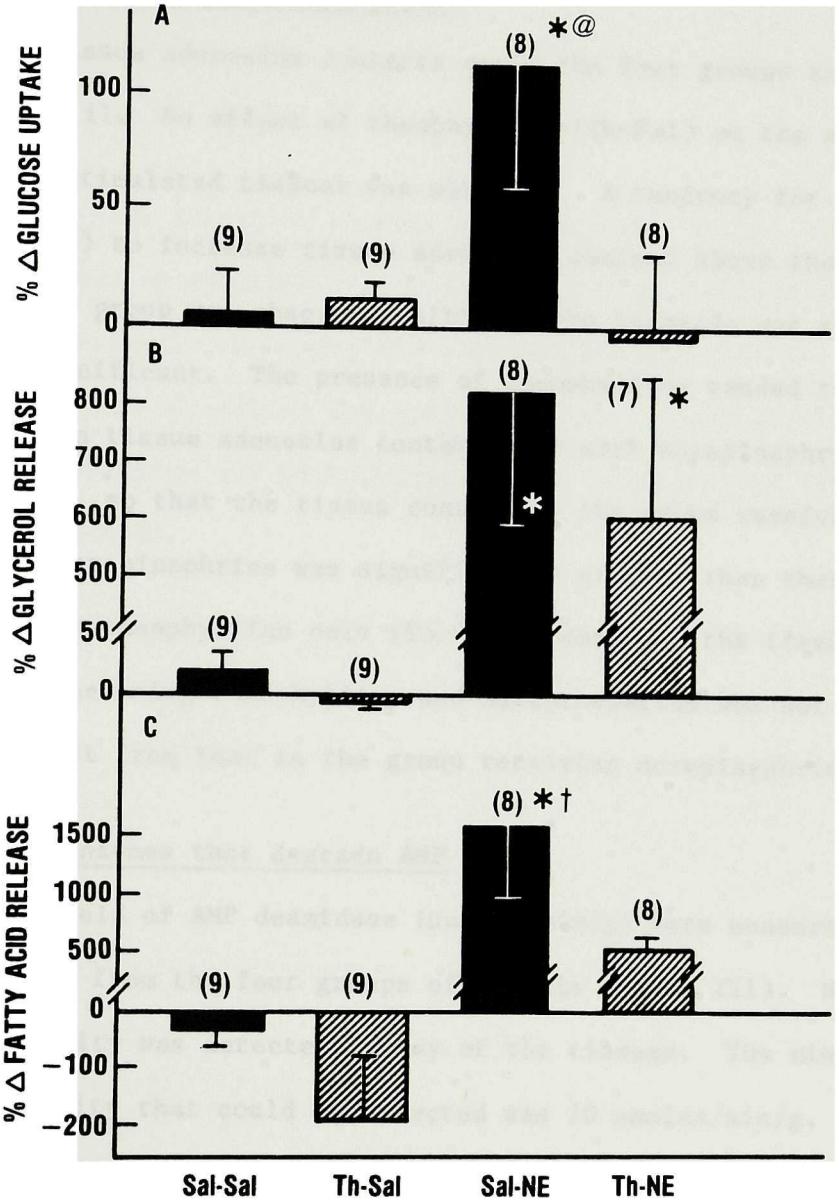

A
B
C
% Δ GLUCOSE UPTAKE
% Δ GLYCEROL RELEASE
% Δ FATTY ACID RELEASE
50
25
0
– 12.5
0
– 25
– 50
0
– 100
– 250
– 350
(13)
(14)
(16)
(17)
(13)
(9)
(15)
(15)
(13)
(13)
(16)
(18)
*
*
*
SAL
TH
SAL
TH
PEAK (13 ′)
BEFORE NE (60 ′)

after saline or theophylline treatment. Fatty acid uptake was significantly increased in saline- as compared to theophylline-treated animals. However, these differences reflect very low absolute releases or uptakes in a few animals, resulting in large calculated percent changes. By 60 min, fatty acid release was similar between the saline- and theophylline-treated groups. These data indicate that after one hour of exposure, theophylline did not significantly inhibit glucose uptake or stimulate basal lipolysis.

Effect of theophylline on norepinephrine-stimulated metabolism

Figure 10 shows the percent changes in metabolic parameters over the time of the norepinephrine infusion (60 min to 80 min). The intravenous infusion of norepinephrine (Sal-NE) significantly increased glucose uptake (panel A) as compared with the saline control group (Sal-Sal). The presence of theophylline (Th-NE) significantly attenuated the increase in glucose uptake observed with norepinephrine stimulation. In fact, the percent change in glucose uptake in the presence of theophylline was similar to that in groups that did not receive norepinephrine. Lipolysis, as measured by either glycerol release (panel B) or fatty acid release (panel C) was significantly increased by intravenous norepinephrine. Theophylline did not potentiate the norepinephrine-stimulated glycerol release (panel B). Rather, theophylline tended to inhibit the norepinephrine-induced increases in glycerol release, although differences are not statistically significant. Theophylline significantly inhibited the norepinephrine-stimulated increase in free fatty acid release (panel C). Over this time period, fatty acid release in the unstimulated groups did not change significantly. These data indicate that during lipolytic stimulation by intravenous norepinephrine, theophylline inhibits glucose

Figure 10. Percent change (%Δ) in metabolic parameters over the time (60 min to 80 min) of an intravenous infusion of saline (-Sal) or norepinephrine (-NE). Values are means± SEM. ( )=number of animals. Sal-Sal=animals that received saline only. Th-Sal=animals that received theophylline and saline. Sal-NE=animals that received saline and norepinephrine. Th-NE=animals that received theophylline and norepinephrine. *=significantly different from Sal-Sal, $P<0.05$. +=significantly different from Th-NE, $P<0.05$. @=significantly different from Th-Sal, $P<0.05$.


A
% Δ GLUCOSE UPTAKE
100
50
0
(9)
(9)
(8) *@
(8)
B
% Δ GLYCEROL RELEASE
800
700
600
500
50
0
(9)
(9)
(8)
*
(7) *
C
% Δ FATTY ACID RELEASE
1500
1000
500
0
−100
−200
(9)
(9)
(8) *†
(8)
Sal-Sal
Th-Sal
Sal-NE
Th-NE

uptake. Theophylline also appears to blunt the lipolytic response to norepinephrine as indicated by the inhibition of the fatty acid release and the tendency for a decrease in the glycerol release.

Tissue adenosine contents among groups

The tissue adenosine contents among the four groups are presented in Figure 11. No effect of theophylline (Th-Sal) on the adenosine content of unstimulated tissues was observed. A tendency for norepinephrine (Sal-NE) to increase tissue adenosine content above that seen in the control group was observed, although the increase was not statistically significant. The presence of theophylline tended to enhance the increase in tissue adenosine content seen with norepinephrine stimulation (Th-NE), so that the tissue content in the group receiving theophylline and norepinephrine was significantly greater than that in the group receiving theophylline only (Th-Sal). However, the tissue content in the group receiving theophylline and norepinephrine was not significantly different from that in the group receiving norepinephrine alone.

Activities of enzymes that degrade AMP

The levels of AMP deaminase (μmoles/min/g) were measured in adipose tissues from the four groups of animals (Table III). No AMP deaminase activity was detected in any of the tissues. The minimal amount of activity that could be detected was 10 μmoles/min/g.

Table III also shows the activities of 5'-nucleotidase measured in the four groups of animals. The activity of 5'-nucleotidase in the group receiving theophylline alone was significantly greater than that in the control group. The activity measured in the group receiving norepinephrine alone was significantly lower than that in control.

Figure 11. Tissue adenosine content among groups. Values are means ±SEM. ( )=number of animals. Sal-Sal=animals that received saline only. Th-Sal=animals that received theophylline and saline. Sal-NE= animals that received saline and norepinephrine. Th-NE=animals that received theophylline and norepinephrine. @=significantly different from Th-Sal, $P<0.05$.

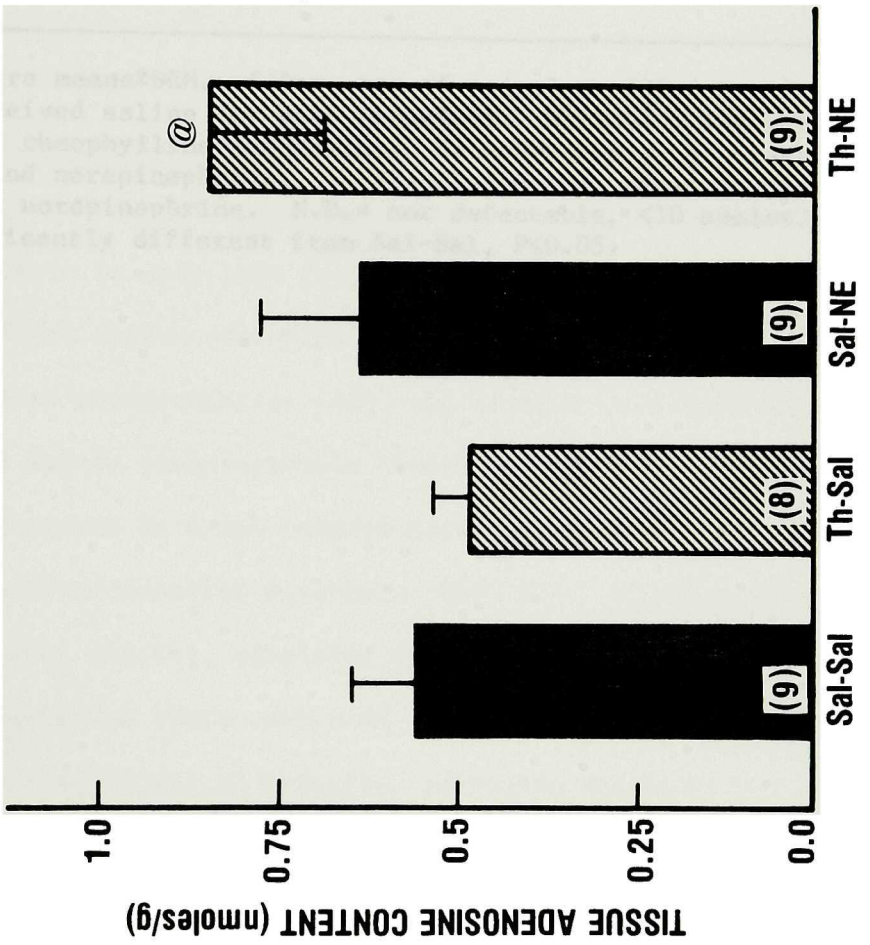

TISSUE ADENOSINE CONTENT (nmoles/g)
1.0
0.75
0.5
0.25
0.0
@
(9)
(8)
(9)
(9)
Sal-Sal
Th-Sal
Sal-NE
Th-NE

Table III. Activities of enzymes that degrade AMP.

Group	AMP deaminase activity (μmole/min/g)	5'-nucleotidase activity (nmole/min/g)
Sal-Sal	N.D. (5)	60±12 (4)
Th-Sal	N.D. (4)	106±3* (3)
Sal-NE	N.D. (5)	18±6* (5)
Th-NE	N.D. (3)	72±9 (3)

Values are means±SEM. ( )=number of animals. Sal-Sal=animals that received saline for both infusions. Th-Sal= animals that received theophylline and saline. Sal-NE=animals that received saline and norepinephrine. Th-NE=animals that received theophylline and norepinephrine. N.D.= not detectable, <10 μmoles/min/g. *=significantly different from Sal-Sal, $P<0.05$.

Enzyme activities were not significantly different between the control group and that group receiving theophylline and norepinephrine. These data indicate the existence of a pathway for the degradation of AMP to adenosine (5'-nucleotidase). An enzymatic pathway for the degradation of AMP to IMP (AMP deaminase) was not detected. Moreover, an effect of theophylline or norepinephrine on 5'-nucleotidase activity *in vivo* was observed.

ADENOSINE DEAMINASE STUDIES

Lymph adenosine deaminase activity

Lymph was collected and assayed for adenosine deaminase activity in three animals that received boiled deaminase (BLD), in three that received active adenosine deaminase (ADA), in two that received Krebs before norepinephrine (NE), and in four that received adenosine deaminase before norepinephrine (ADA/NE). Adenosine deaminase activity was not detected in lymph samples collected from animals that did not receive active adenosine deaminase (BLD, NE). In the deaminase-treated groups, (ADA, ADA/NE), adenosine deaminase activity was not detected in the samples of lymph collected before the infusion of adenosine deaminase. By 30 min of infusion, adenosine deaminase was detected in the lymph from all animals receiving an infusion of adenosine deaminase.

Hemodynamics

At the onset of the procedure, groups were similar with respect to mean arterial blood pressure and blood flow (Table IV). Blood flows in individual animals ranged from 5.3 to 12.3 ml/min/100g. Blood pressures were stable over the 90 min procedure for the unstimulated groups (BLD, ADA) and over the 65 min procedure for the norepinephrine-stimu-

Table IV. Initial and final hemodynamic measurements in the adenosine deaminase groups.

Initial measurements (0 min)

Group	MABP (mmHg)	Q (ml/min/ 100g)	VR (mmHg·min· 100g)/ml	pH	PO_2 (mmHg)	PCO_2 (mmHg)	Hct (%)
BLD	141 ±2	7.6 ±0.6	18.8 ±1.3	7.40 ±0.01	96 ±2	34 ±1	42 ±2
ADA	140 ±9	9.4 ±0.8	15.8 ±1.9	7.37 ±0.01	94 ±3	36 ±1	40 ±2
NE	144 ±5	7.2 ±0.8	21.3 ±2.4	7.39 ±0.01	97 ±4	38 ±1	41 ±1
ADA/NE	138 ±5	8.8 ±0.7	16.3 ±1.8	7.39 ±0.01	102 ±4	37 ±1	41 ±2

Final measurements (90 min for BLD, ADA; 65 min for NE, ADA/NE)

Group	MABP (mmHg)	Q (ml/min/ 100g)	VR (mmHg·min· 100g)/ml	pH	PO_2 (mmHg)	PCO_2 (mmHg)	Hct (%)
BLD	143 ±3	5.4 ±1.1	31.8 ±6.6	7.39 ±0.01	95 ±2	34 ±1	41 ±2
ADA	139 ±9	4.2 ±0.4	35.5 ±4.2	7.39 ±0.01	92 ±2	34 ±1	42 ±2
NE	144 ±9	6.3 ±0.9	25.7 ±3.4	7.36 ±0.01	97 ±4	39 ±2	47 ±2
ADA/NE	138 ±5	6.8 ±0.9	23.3 ±3.8	7.36 ±0.01	102 ±3	37 ±1	47 ±3

Values are means±SEM.
MABP=mean arterial blood pressure
Q=blood flow per 100g tissue
VR=vascular resistance
pH, PO_2, PCO_2=arterial blood pH and gases
Hct=hematocrit

BLD=control, boiled deaminase
ADA=active adenosine deaminase
NE=norepinephrine
ADA/NE=adenosine deaminase/ norepinephrine

lated groups (NE, ADA/NE). The intravenous infusion of norepinephrine (0.25 μg/kg/min; NE, ADA/NE) transiently increased mean arterial blood pressure. However, after 10 min of norepinephrine infusion, blood pressures had returned to pre-norepinephrine levels. At this lower dose of norepinephrine, less effect on pH and hematocrit was observed as compared with the theophylline series. Arterial blood gases in all groups were within physiologic limits over the procedure. Tissue weights ranged from 36 to 98 g, with an average of 63±3 g for the series.

Effect of adenosine deaminase on vascular resistance

Because of individual variation in vascular resistance, the effects of the intraarterial infusion of either vehicle or active enzyme on vascular resistance are expressed as percent changes from the resistance at the onset of the infusion. The infusion of adenosine deaminase caused an immediate, transient vasodilation that lasted up to 10 min. In unstimulated tissues, by 30 min of infusion, adenosine deaminase (ADA) had significantly increased vascular resistance as compared with control (BLD, Figure 12). Adenosine deaminase continued to increase vascular resistance significantly over the remainder of the infusion to a greater extent than that increase expected with time alone (BLD).

Effect of adenosine deaminase on the vascular response to norepinephrine

The percent changes in vascular resistance over the time of the intravenous infusion of norepinephrine are presented in Figure 13. Vasodilation was observed by 9 min of norepinephrine infusion (NE). After 12 min of norepinephrine infusion, a significantly greater decrease in vascular resistance was observed as compared with the response at 3 min.

Figure 12. Percent change (%Δ) in vascular resistance over the time of an intraarterial infusion of boiled (BLD) or active adenosine deaminase (ADA) under unstimulated conditions. Values are means±SEM. ( )=number of animals. *=significantly different from BLD at that time, $P<0.05$.

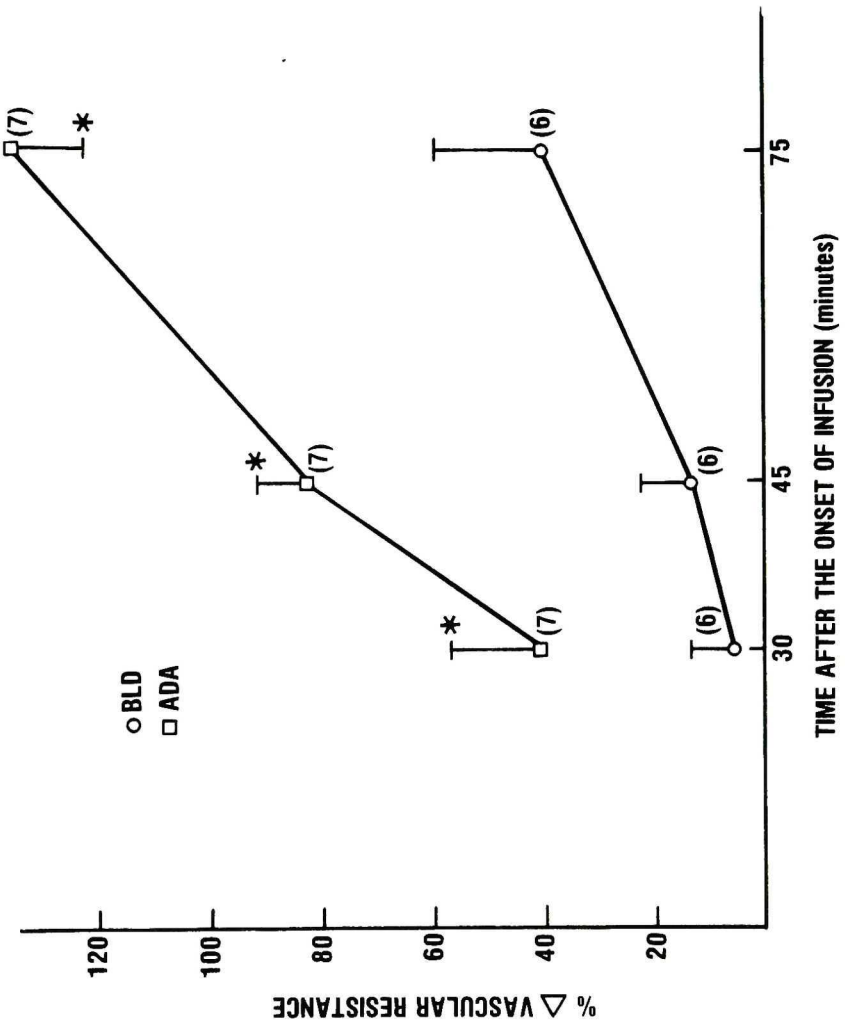
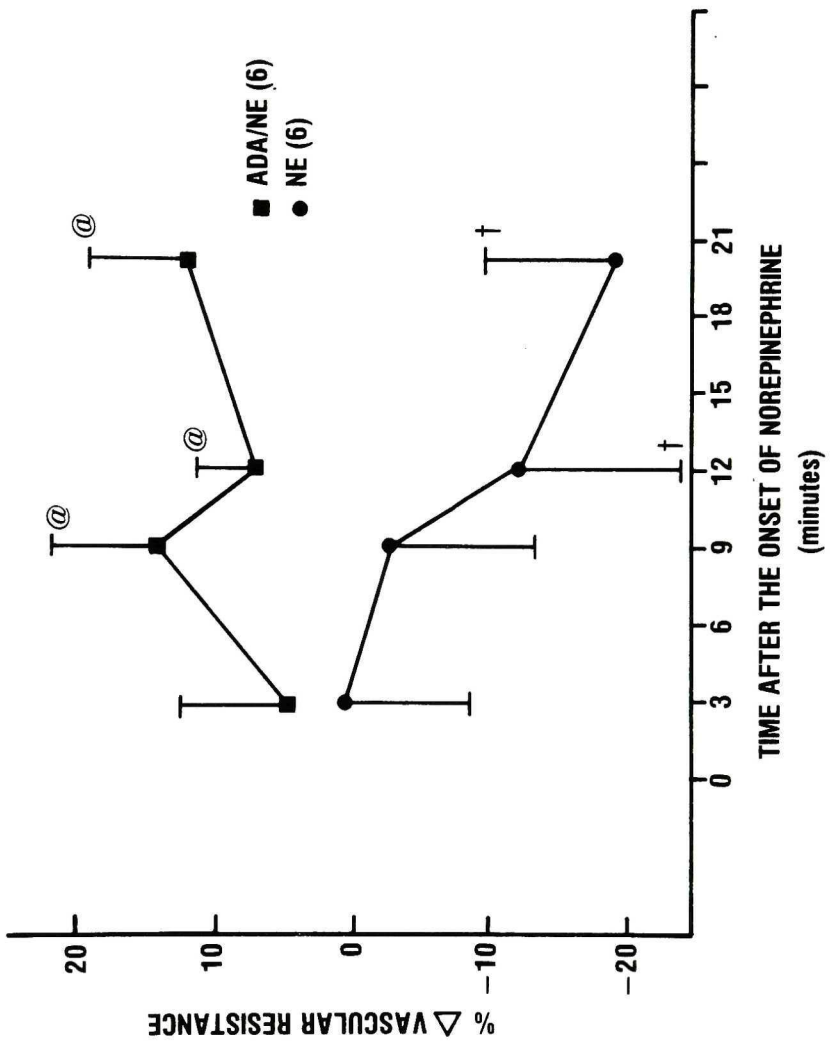

% Δ VASCULAR RESISTANCE
120
100
80
60
40
20
30
45
75
TIME AFTER THE ONSET OF INFUSION (minutes)
○ BLD
□ ADA
(7)
(7)
(7)
(6)
(6)
(6)
*
*
*

Figure 13. Vascular response to intravenous norepinephrine in the absence (NE) or presence (ADA/NE) of adenosine deaminase. Values are means±SEM. ( )=number of animals. @=significantly different from NE at that time, $P<0.05$. +=significantly different from the response at 3 min, $P<0.05$.


ADA/NE (6)
NE (6)
@
@
@
†
†
20
10
0
−10
−20
% Δ VASCULAR RESISTANCE
0
3
6
9
12
15
18
21
TIME AFTER THE ONSET OF NOREPINEPHRINE
(minutes)

Vascular resistance continued to decrease over the remainder of the norepinephrine infusion. The prior treatment of the tissue with adenosine deaminase completely blocked the vasodilation observed with norepinephrine alone. As measured by percent change, at 9 min, 12 min, and 20 min, the resistance in the deaminase-treated group (ADA/NE) was significantly increased as compared to that in the norepinephrine (NE) group. At no time point was the mean vascular resistance decreased in the deaminase-treated group.

Figure 14 illustrates the percent changes in vascular resistance in the unstimulated groups and the norepinephrine-treated groups. The changes in resistance were calculated over the time corresponding to the time of the norepinephrine infusion. The data indicate that the presence of adenosine deaminase alone significantly increased vascular resistance in basal, unstimulated tissue. The vasodilation seen in tissues in which lipolysis had been stimulated with norepinephrine was completely blocked by adenosine deaminase. However, in this group (ADA/NE), vascular resistance did not increase to the extent observed in the group that received adenosine deaminase alone (ADA).

Effect of adenosine deaminase on unstimulated metabolism

The absolute values for glucose uptake and glycerol release among groups at the onset of the procedure are shown in Table V. Although initial measurements were variable, groups were not significantly different with respect to metabolic indices. Metabolic measurements during the initial vasodilation caused by adenosine deaminase were not significantly different from those before the start of the intraarterial infusion. By 45 min of infusion, in unstimulated tissues, glucose uptake was significantly decreased by adenosine deaminase as compared with control (BLD; Figure 15, panel A). An effect of time on

Figure 14. Percent change (%Δ) in vascular resistance over the time of an intravenous infusion of norepinephrine (45 min to 65 min) in the stimulated groups (NE, ADA/NE) or over a similar time period (45 min to 60 min) in the unstimulated groups (BLD, ADA). Values are means±SEM. ( )=number of animals. BLD=animals that received boiled deaminase. ADA=animals that received adenosine deaminase. NE=animals that received Krebs and norepinephrine. ADA/NE=animals that received adenosine deaminase and norepinephrine. *=significantly different from BLD, $P<0.05$. @=significantly different from ADA/NE, $P<0.05$.

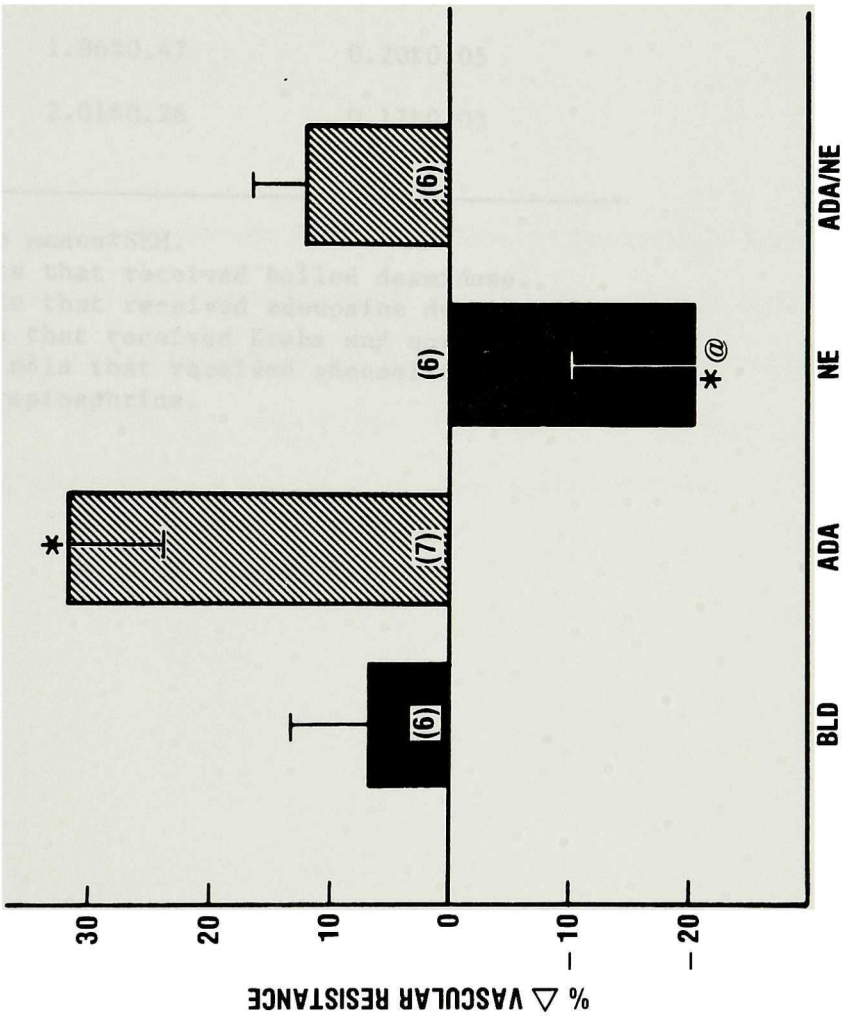
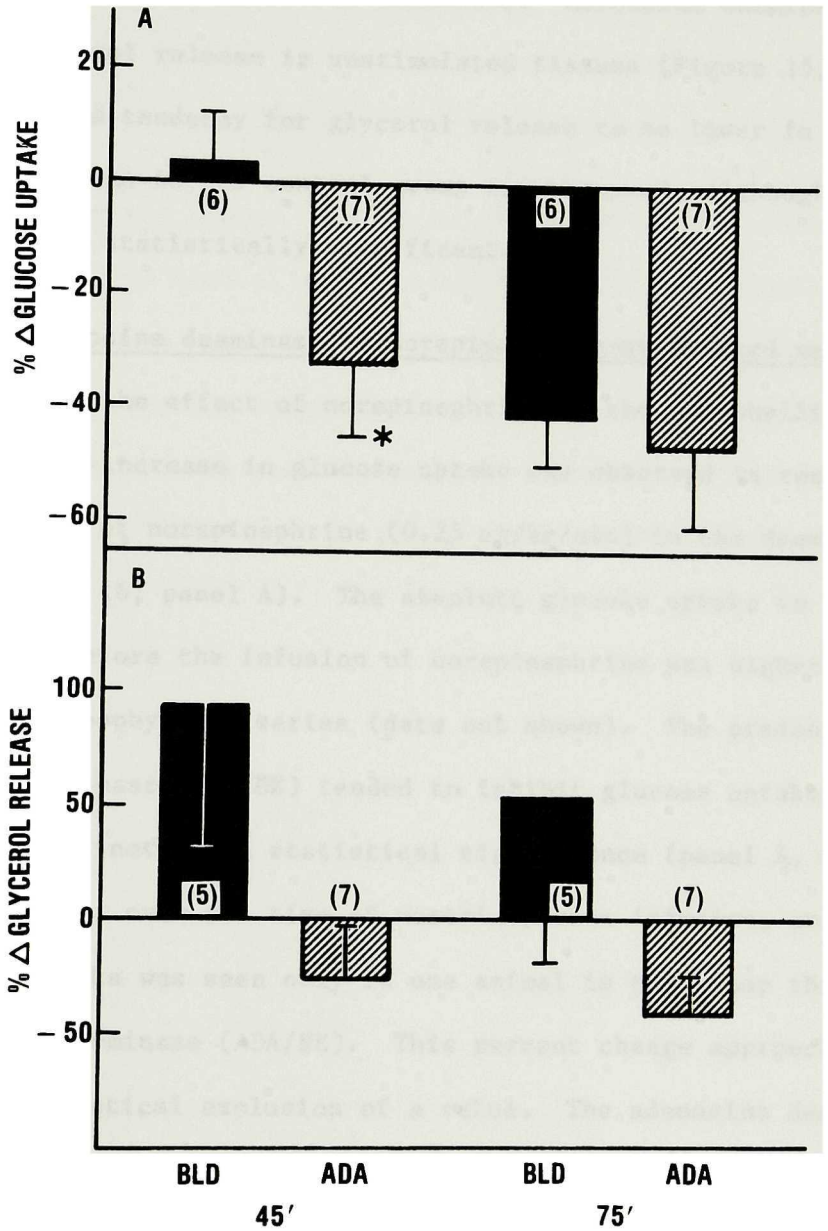

% ∆ VASCULAR RESISTANCE
30
20
10
0
−10
−20
BLD
ADA
NE
ADA/NE
(6)
(7)
(6)
(6)
*
*@

Table V. Initial metabolic measurements in the adenosine deaminase groups.

Group	Glucose Uptake (μmole/min/100g)	Glycerol Release (μmole/min/100g)
BLD	1.48±0.28	0.09±0.03
ADA	1.76±0.35	0.07±0.01
NE	1.96±0.47	0.20±0.05
ADA/NE	2.01±0.26	0.17±0.03

Values are means±SEM.
BLD=animals that received boiled deaminase.
ADA=animals that received adenosine deaminase.
NE=animals that received Krebs and norepinephrine.
ADA/NE=animals that received adenosine deaminase and norepinephrine.

Figure 15. Percent change (%Δ) in metabolic parameters measured at 45 min and at 75 min from the onset of an intraarterial infusion of boiled (BLD) or active adenosine deaminase (ADA) under unstimulated conditions. Values are means±SEM. ( )=number of animals. *=significantly different from BLD at that time point, $P<0.05$.


A
20
0
−20
−40
−60
% Δ GLUCOSE UPTAKE
(6)
(7)
(6)
(7)
*
B
100
50
0
− 50
% Δ GLYCEROL RELEASE
(5)
(7)
(5)
(7)
BLD
ADA
BLD
ADA
45′
75′

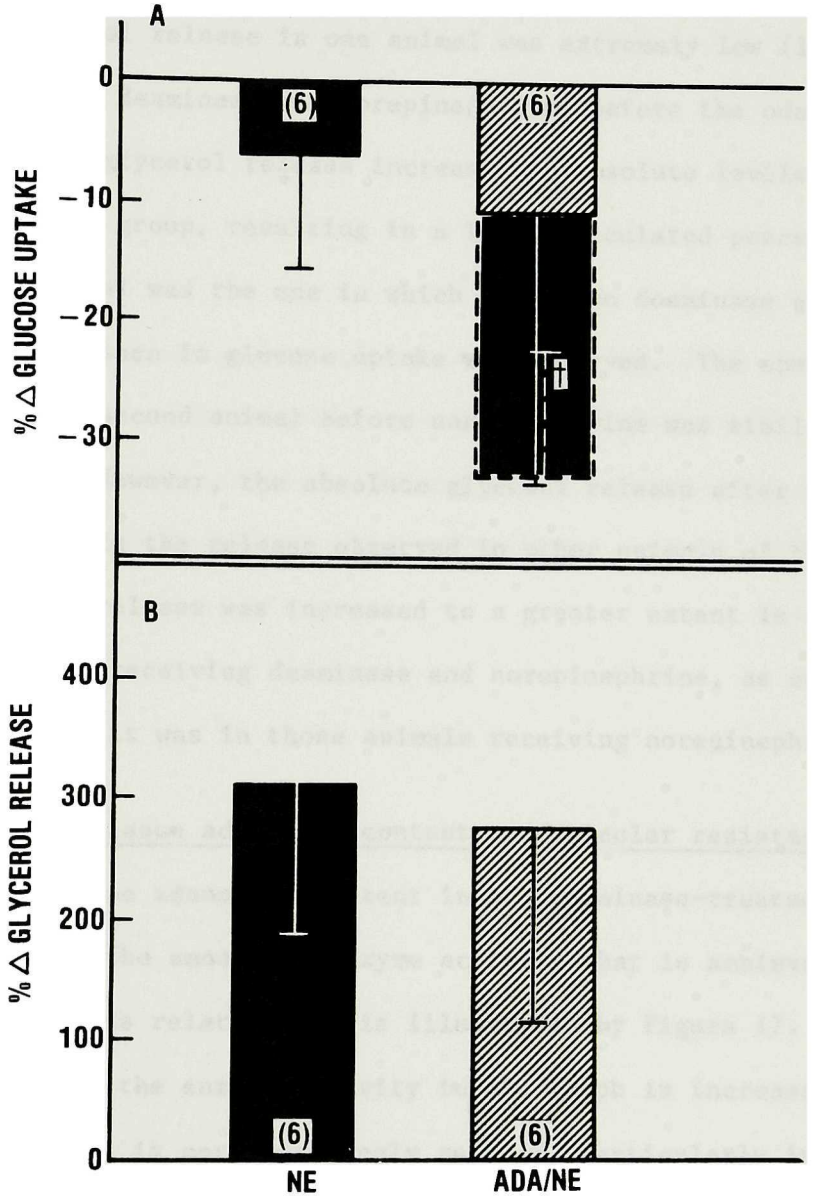
glucose uptake was observed; by 75 min, glucose uptake declined in in the control group and was not significantly different from that in the deaminase-treated group. Glucose uptake was the only metabolic parameter affected by adenosine deaminase. Adenosine deaminase did not increase glycerol release in unstimulated tissues (Figure 15, panel B). In fact, a tendency for glycerol release to be lower in the deaminase-treated than in the control group was observed, although the effect was not statistically significant.

Effect of adenosine deaminase on norepinephrine-stimulated metabolism

Unlike the effect of norepinephrine in the theophylline series, no significant increase in glucose uptake was observed in response to the lower dose of norepinephrine (0.25 μg/kg/min) in the deaminase series (Figure 16, panel A). The absolute glucose uptake in the deaminase series before the infusion of norepinephrine was higher than that in the theophylline series (data not shown). The presence of adenosine deaminase (ADA/NE) tended to inhibit glucose uptake, but the differences did not reach statistical significance (panel A, solid line). However, over the time of norepinephrine infusion, an increase in glucose uptake was seen only in one animal in the group that was treated with deaminase (ADA/NE). This percent change approached the point for statistical exclusion of a value. The adenosine deaminase activity in this animal was the lowest measured in any deaminase-treated animal. When the glucose uptake in this group was considered in the absence of this one animal, a signficant inhibition of glucose uptake was observed (panel A, dotted line).

On the average, adenosine deaminase did not significantly affect the increase in glycerol release elicited by norepinephrine (Figure 16,

Figure 16. Percent change (%Δ) in metabolic parameters over the time (45 min to 65 min) of an intravenous infusion of norepinephrine. Values are means±SEM. ( )=number of animals. NE=animals that received Krebs and norepinephrine. ADA/NE=animals that received adenosine deaminase and norepinephrine. +=the dotted line represents all but one animal (N=5) and is significantly different from NE, $P<0.05$.

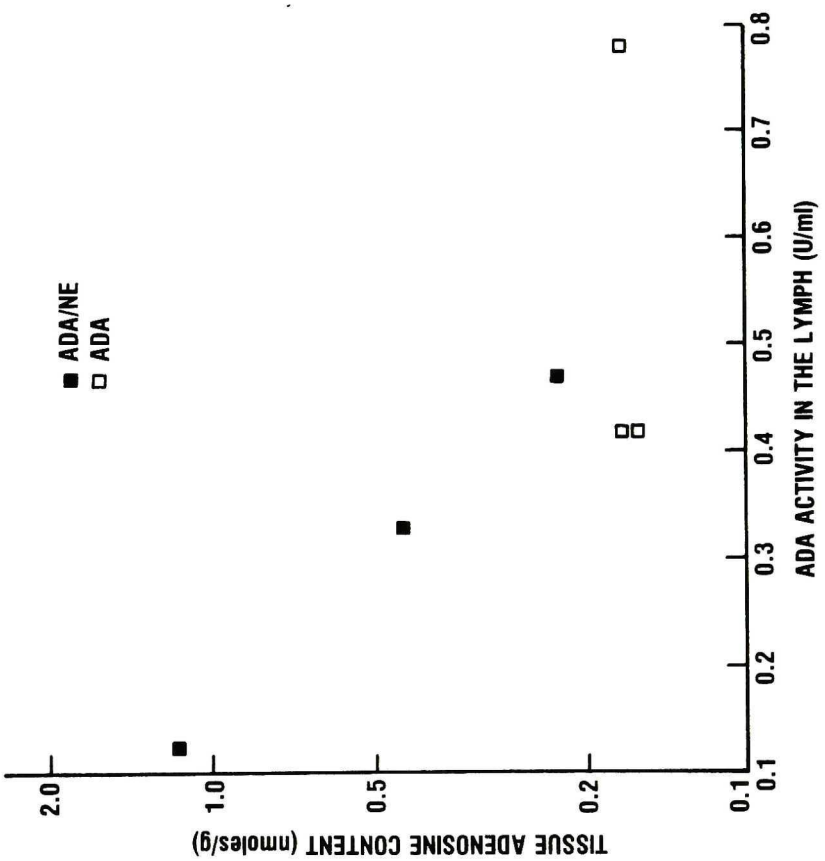

A
0
−10
−20
−30
% Δ GLUCOSE UPTAKE
(6)
(6)
B
400
300
200
100
0
% Δ GLYCEROL RELEASE
(6)
(6)
NE
ADA/NE

panel B). However, in two animals in this group, an apparent stimulation of lipolysis was observed. The percent changes in glycerol release were 1650% and 1000%. These percent changes in glycerol release were the largest observed in any of the norepinephrine-treated animals. The absolute glycerol release in one animal was extremely low (lowest of the group receiving deaminase and norepinephrine) before the onset of norepinephrine. The glycerol release increased to absolute levels similar to the rest of the group, resulting in a large calculated percent change. This first animal was the one in which adenosine deaminase activity was low and an increase in glucose uptake was observed. The absolute glycerol release in the second animal before norepinephrine was similar to others in the group. However, the absolute glycerol release after norepinephrine was almost double the release observed in other animals of the group. Thus, glycerol release was increased to a greater extent in only one of the six animals receiving deaminase and norepinephrine, as compared to the extent that it was in those animals receiving norepinephrine alone.

Correlation of tissue adenosine content and vascular resistance

The tissue adenosine content in the deaminase-treated animals is dependent on the amount of enzyme activity that is achieved in the lymph fluid. This relationship is illustrated by Figure 17. The data indicate that as the enzyme activity in the lymph is increased, the adenosine content is correspondingly reduced, particularly in the presence of norepinephrine. As observed in Figure 17, to achieve a tissue content of adenosine in the norepinephrine-stimulated (ADA/NE) group that approaches that in the basal, deaminase (ADA) group, the adenosine deaminase activity (ADA/NE) should reach approximately 0.3 μmoles/min/ml. In one animal receiving deaminase and norepinephrine

Figure 17. Tissue adenosine content as a function of adenosine deaminase activity. Each point represents a single animal. ADA= animals that received adenosine deaminase. ADA/NE=animals that received adenosine deaminase and norepinephrine. U/ml= μmoles/min/ml.


■ ADA/NE
□ ADA
TISSUE ADENOSINE CONTENT (nmoles/g)
2.0
1.0
0.5
0.2
0.1
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
ADA ACTIVITY IN THE LYMPH (U/ml)

in which the deaminase activity was only 0.13 μmole/min/ml, the tissue adenosine content was greater than 1 nmole/g, indicating that deaminase activity was not sufficient to degrade interstitial adenosine.

The tissue adenosine contents for the four groups are presented in Figure 18. Infusion of adenosine deaminase into basal tissues reduced the adenosine content to approximately 50% of that in the control group. The adenosine content in one of the animals receiving adenosine deaminase was much greater than that in the others. No lymph samples were collected, so the level of deaminase that was achieved is not known. When the average of the group (Figure 18, dotted line, ADA) is considered without this value, adenosine deaminase significantly decreased the tissue adenosine content as compared with that in the control group. The infusion of norepinephrine increased tissue adenosine content, although the increase was not significantly different from control. In five of six animals, adenosine deaminase was able to degrade a large portion (50%) of the hormone-stimulated adenosine content, so that levels approached those in unstimulated, control tissues (BLD). In the sixth animal, adenosine deaminase activity was low (0.13 μmoles/min/ml). Tissue adenosine content in this animal was as high as that observed in the group receiving norepinephrine alone. When the average is calculated without this one animal (dotted line, ADA/NE), the infusion of adenosine deaminase significantly reduced the tissue adenosine content as compared with the group receiving norepinephrine alone.

A significant inverse relationship between tissue adenosine content and vascular resistance was observed among the control group, the group that received deaminase alone, and the group that received norepinephrine alone (Figure 19). Although a significant inverse relation-

Figure 18. Tissue adenosine contents among groups. Values are means±SEM. ( )=number of animals. BLD=animals that received boiled deaminase. ADA= animals that received adenosine deaminase. NE=animals that received norepinephrine. ADA/NE=animals that received adenosine deaminase and norepinephrine. @=significantly different from NE, $P<0.05$. *=the dotted line represents all but one animal (N=6) and is significantly different from BLD, $P<0.05$. +=the dotted line represents all animals except one in which adenosine deaminase activity was extremely low (N=4) and is significantly different from NE, $P<0.05$.

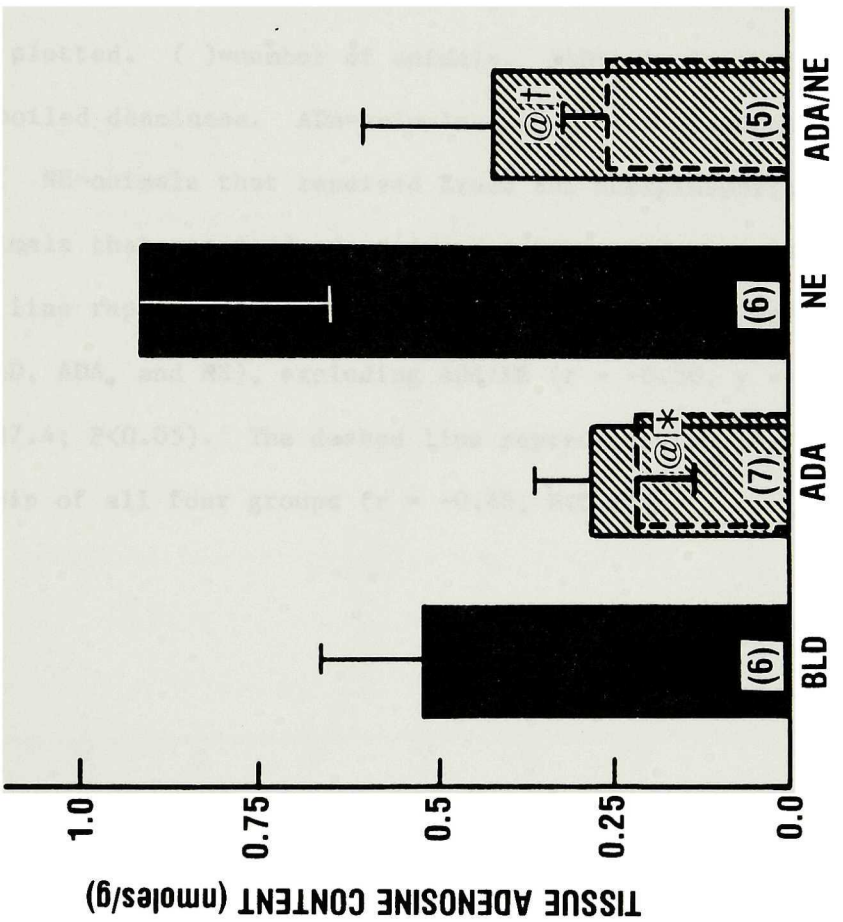
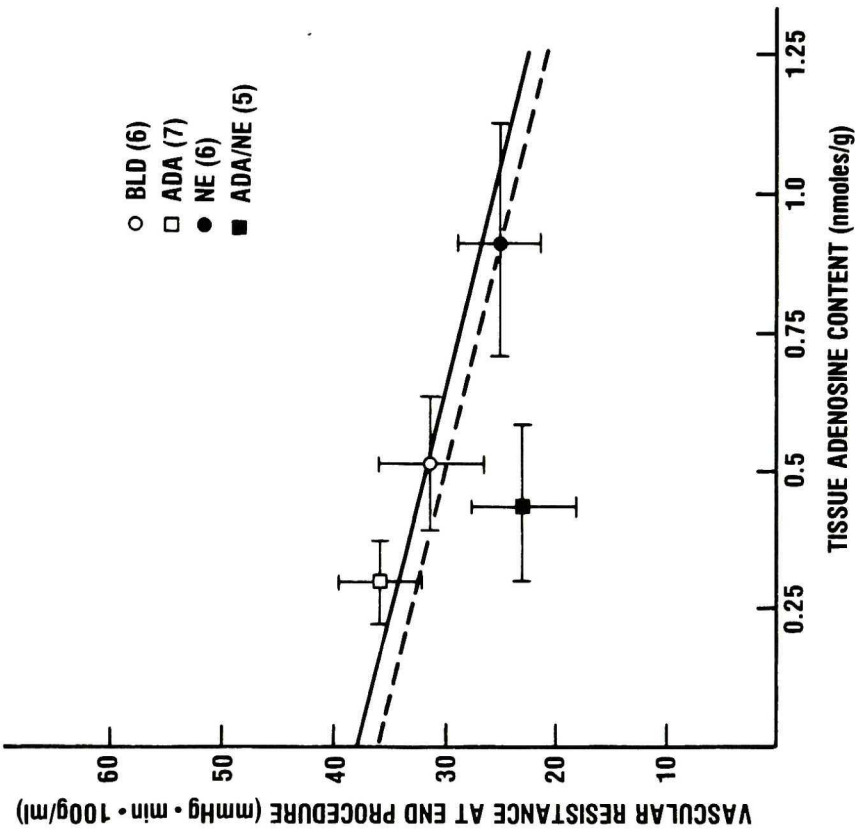

TISSUE ADENOSINE CONTENT (nmoles/g)
1.0
0.75
0.5
0.25
0.0
(6)
BLD
@*
(7)
ADA
(6)
NE
@†
(5)
ADA/NE

Figure 19. Vascular resistance at the end of the procedure as a function of tissue adenosine content. The means±SEM for each group are plotted. ( )=number of animals. BLD=animals that received boiled deaminase. ADA=animals that received adenosine deaminase. NE=animals that received Krebs and norepinephrine. ADA/NE=animals that received adenosine deaminase and norepinephrine. The solid line represents the regression relationship among three groups (BLD, ADA, and NE), excluding ADA/NE ($r = -0.50$, $y = -11.2x + 37.4$; $P<0.05$). The dashed line represents the regression relationship of all four groups ($r = -0.46$, $P<0.05$).

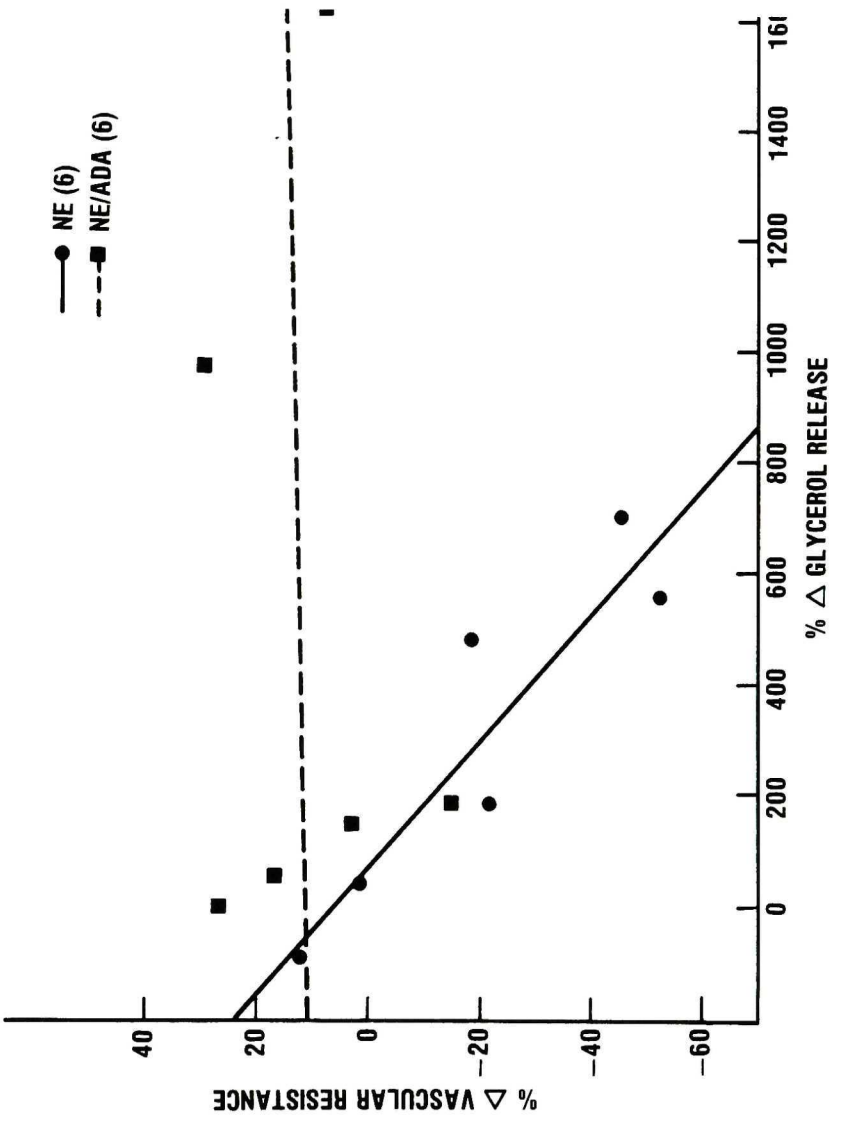

○ BLD (6)
□ ADA (7)
● NE (6)
■ ADA/NE (5)
VASCULAR RESISTANCE AT END PROCEDURE (mmHg • min • 100g/ml)
60
50
40
30
20
10
0.25
0.5
0.75
1.0
1.25
TISSUE ADENOSINE CONTENT (nmoles/g)

ship ($r = -0.46$, $P<0.05$; $N=24$) is observed when all animals from all groups are considered, the average vascular resistance in the group that received deaminase and norepinephrine (ADA/NE) falls below that predicted by the relationship among the other three groups. Three of the animals receiving deaminase and norepinephrine fall on the line calculated for the other groups. The remaining two animals in this group fall below the line. Vascular resistance in these two animals was low at the onset of the procedure. Norepinephrine did not decrease vascular resistance in these two animals. These data indicate that alterations in tissue adenosine content, whether adenosine is decreased by adenosine deaminase or increased by norepinephrine, affect the vascular resistance in adipose tissue.

Correlation of glycerol release and vascular resistance

Figure 20 shows the inverse relationship between lipolytic (as measured by glycerol release) and vascular events over the time of the norepinephrine infusion. The solid regression line illustrates the norepinephrine group only and indicates that the more intense the metabolic stimulation, the more pronounced the vasodilation. The points representing the animals receiving deaminase and norepinephrine are plotted and indicate that in four animals, the predicted relationship is maintained. However, these four points lie toward the upper left area of the graph, indicating that the presence of adenosine deaminase blocks the vasodilation elicited by norepinephrine and tends to blunt the change in glycerol release. The removal of adenosine seems to alter the association between glycerol release and vascular resistance in two animals that were described previously. In these two animals, glycerol release appears to be potentiated by the combination of adeno-

Figure 20. The percent change (%Δ) in vascular resistance as a function of the percent change (%Δ) in glycerol release over the time of the norepinephrine infusion (45 min to 65 min). Each point represents a single animal for each of the stimulated groups (NE and ADA/NE). NE=animals that received Krebs and norepinephrine. ADA/NE=animals that received adenosine deaminase and norepinephrine. The solid line represents the regression relationship in NE, $P<0.02$ ($r = -0.91$, $y = -0.1x + 2.4$). The dashed line represents the regression relationship in ADA/NE, $P>0.1$ ($r = 0.11$, $y = .003x + 10.5$).

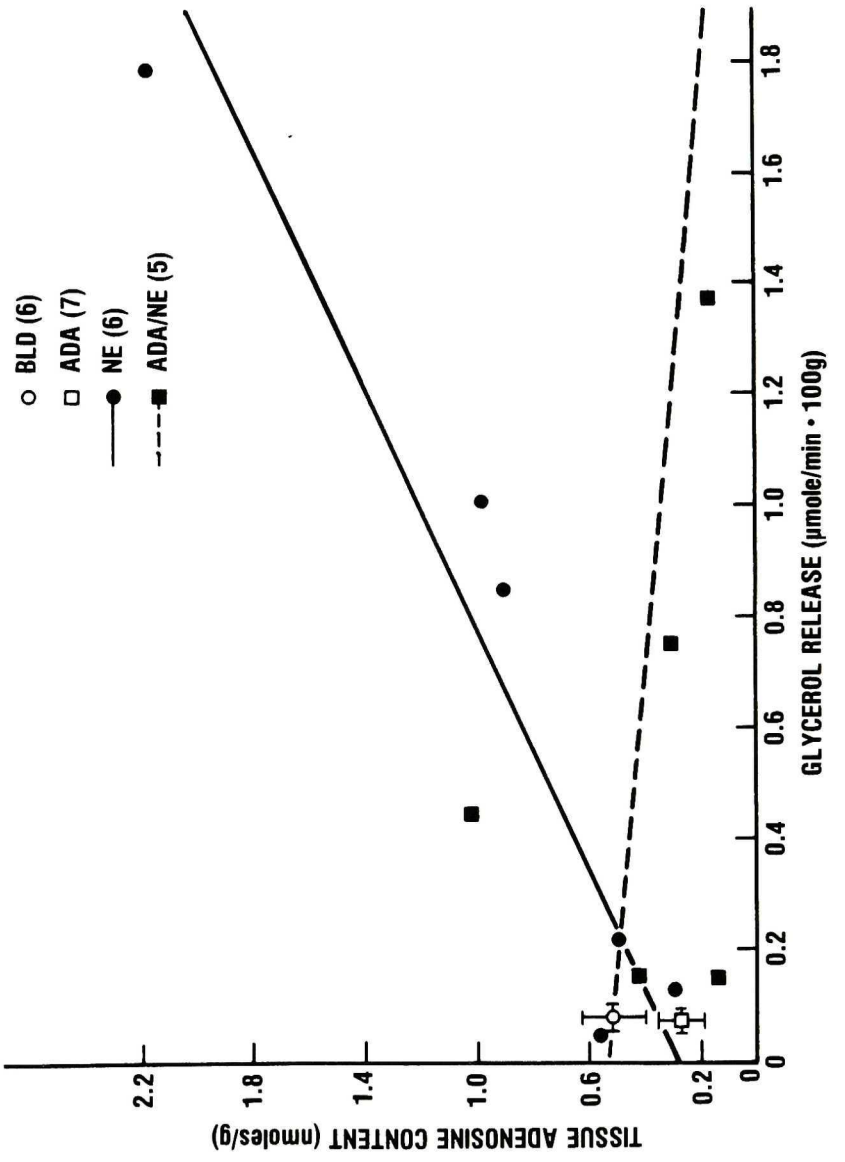

NE (6)
NE/ADA (6)
% Δ VASCULAR RESISTANCE
40
20
0
−20
−40
−60
0
200
400
600
800
1000
1200
1400
% Δ GLYCEROL RELEASE

sine deaminase and norepinephrine. However, in one animal, the percent change reflects a very low pre-stimulation value, rather than a large increase in glycerol release with norepinephrine. The glycerol release in the second animal was greatly increased by norepinephrine. The adenosine deaminase activity in this animal was very low, indicating that interstitial levels of adenosine would be higher than those in the rest of the group. However, in both animals, no vasodilation accompanied the increased metabolism. These data indicate that the metabolic and vascular responses to norepinephrine are diminished in the presence of adenosine deaminase.

Correlation of glycerol release and tissue adenosine content

The relationship between tissue adenosine content and glycerol release is illustrated in Figure 21. Because glycerol release was very low and did not vary much in the unstimulated groups, the relationship is best examined in tissues in which lipolysis has been stimulated. The mean data for the unstimulated groups are plotted but are not included in the calculation of the regression line. A significant relationship is observed in animals receiving norepinephrine alone (solid line). The presence of adenosine deaminase dissociates the relationship (dashed) line). Although absolute levels of glycerol release are similar with the infusion of norepinephrine in both groups, the tissue content of adenosine is lower and relatively constant in the deaminase and norepinephrine group as compared with the group that received norepinephrine alone. Figure 21 also illustrates that one animal that received deaminase and norepinephrine had a high tissue adenosine content. The enzyme activity in this animal was the lowest detected in any deaminase-treated animal. These data indicate that norepinephrine-stimulated lipolysis is associated with an increased tissue adenosine content.

Figure 21. Tissue adenosine content as a function of glycerol release at the end of the procedure. Values for the means±SEM of the unstimulated groups are plotted (BLD and ADA). ( )=number of animals. BLD=animals that received boiled deaminase. ADA= animals that received adenosine deaminase. NE=animals that received Krebs and norepinephrine. ADA/NE=animals that received adenosine deaminase and norepinephrine. The solid line represents the regression relationship in NE, $P<0.01$ ($r = 0.95$, $y = 0.9x + 0.2$). The dashed line represents the regression relationship in ADA/NE, $P>0.1$ ($r = 0.11$, $y = -0.1x + 11.2$).


○ BLD (6)
□ ADA (7)
● NE (6)
■ ADA/NE (5)
TISSUE ADENOSINE CONTENT (nmoles/g)
2.2
1.8
1.4
1.0
0.6
0.2
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
GLYCEROL RELEASE (µmole/min • 100g)

DISCUSSION

Our studies with adenosine deaminase support the hypothesis that adenosine is a regulator of blood flow in adipose tissues under basal conditions and when lipolysis has been stimulated by the intravenous infusion of norepinephrine. In addition to its role in the regulation of blood flow, we have evidence that adenosine stimulates glucose uptake *in vivo*. However, adenosine does not appear to inhibit basal or catecholamine-stimulated lipolysis *in vivo*, in contrast to the effects reported *in vitro* (Schwabe *et al.*, 1973; Ohisalo, 1981; Turpin *et al.*, 1977).

Our preparations of isolated, subcutaneous adipose tissue of dogs began at comparable hemodynamic and metabolic points as other preparations (McCoy, 1980; Sollevi and Fredholm, 1981b; Sollevi and Fredholm, 1983). The range of blood flows at the onset of the procedure were similar to those reported by others (McCoy, 1980; Sollevi and Fredholm, 1981b). Initial values for glucose uptake and for glycerol release were also within the range of those reported in the literature (McCoy, 1980). Vascular and metabolic measurements were highly variable, a well-documented characteristic of adipose tissue (Rosell and Belfrage, 1979; McCoy, 1980). The variability is not unexpected, considering the multitude of neural, nutritional, and hormonal factors that influence the tissue (Newsholme and Start, 1973).

The infusion of adenosine deaminase increased vascular resistance and decreased tissue adenosine content in basal, unstimulated tissues, indicating that the degradation of endogenous adenosine is associated with a decrease in blood flow. This study is the first that we are aware of to demonstrate an effect of adenosine deaminase on vascular resistance in adipose tissue. Several factors validate the use

of adenosine deaminase to degrade adenosine specifically. Infusion of adenosine deaminase led to increased enzyme activity in the lymph (interstitial space). The activity was inversely related to the tissue adenosine content. Proctor and Duling (1982) reported that, in an *in vitro* preparation of the microvasculature of hamster cremaster muscle, the effects of adenosine deaminase were reversible and did not directly alter the contractile behavior of the vessels. Their data indicate that the effects of adenosine deaminase on vascular resistance were specific to the degradation of adenosine. In addition, in our study, a relatively pure enzyme preparation was achieved, as evidenced by the lack of hemodynamic effects of the heat-denatured (boiled) preparation (no nonprotein contaminant). Gewirtz *et al.* (1983) report that the same commercial product (Sigma, Type I), when chromatographed, elutes as a single peak, further substantiating the purity of the deaminase. Our studies indicate a cause-and-effect relationship between changes in tissue adenosine content and changes in vascular resistance in unstimulated adipose tissue, supporting a role of adenosine in the regulation of blood flow.

In contrast to others (Sollevi and Fredholm, 1981b; Sollevi and Fredholm, 1983), we were unable to show blockade of exogenous adenosine by theophylline (10 mg/kg, i.v.) for up to 60 min of exposure. Sollevi and Fredholm (1981b) did not state at what time after theophylline they tested the vascular effects of adenosine. Therefore, comparisons with the present study are not possible. However, we observed a trend for an increase in vascular resistance with theophylline over 60 min to 80 min of exposure as compared with saline. The delay in the onset of vasoconstriction with theophylline may indicate an effect of theophylline

on intracellular metabolism as opposed to antagonism of adenosine. Our failure to show an effect of theophylline cannot be attributed to the plasma levels of theophylline. We achieved levels of theophylline (30.6±6.1 μM, N=3) at 80 min that were similar to levels (30-100 μM) reported at 30 min by Sollevi and Fredholm (1981b). We were also unable to achieve a blockade of adenosine with intraarterial infusion of theophylline. Our preliminary studies indicate that 10^{-5} M was the maximal plasma concentration that did not cause direct vasodilation. Studies of microvascular preparations have indicated that higher doses (10^{-5} to 10^{-4} M) of theophylline antagonized adenosine only after exposure of the vessels for a minimum of 20 min, and that often, the vessels were visibly damaged (Proctor, 1984). In the present study, theophylline did not increase vascular resistance in unstimulated tissues after 80 min of exposure, indicating that theophylline did not antagonize endogenous adenosine under the conditions of our experiments. In as much as theophylline does not block the vascular actions of exogenous adenosine, our failure to elicit an increase in vascular resistance with theophylline is not unexpected. In view of the findings of the deaminase studies, we conclude that adenosine is a regulator of vascular resistance and that theophylline is not an appropriate antagonist of the vascular actions of adenosine in unstimulated adipose tissue of dogs.

In any proposed theory of metabolic regulation of blood flow, the vasodilator metabolite should be shown to mediate vascular responses in both unstimulated (basal) tissues and in tissues in which metabolism (lipolysis) has been induced. Over a wide range of metabolic activities, the variations in blood flow should be correlated with variations in

the vasodilator. We found that the degradation of endogenous adenosine by adenosine deaminase prevented the vasodilation caused by intravenous norepinephrine (Figures 13 and 14). The hyperemia caused by norepinephrine is proposed to be related to an increase in the metabolism of the tissue (McCoy, 1980; Nielsen et al., 1968), analagous to active hyperemia in the coronary circulation. When lipolysis occurs, the increased release of free fatty acids becomes increasingly dependent on a sufficient supply of albumin (Bulow and Madsen, 1981; Rodbell, 1965; Scow et al., 1965). If the delivery of albumin is impaired, lipolysis is inhibited by the accumulation of free fatty acids (Angel et al., 1971; Bihler and Jeanrenaud, 1970; Rodbell, 1965). Our results indicate that lipolysis, as measured by glycerol release, is strongly correlated with tissue adenosine content (Figure 21). Both the lipolytic response and the tissue adenosine content are strongly correlated with the vascular response, indicating that the greater the metabolic activity, the greater the production of adenosine, and the greater the vascular response. Our results support the hypothesis that the vascular events in adipose tissue are triggered by the level of metabolism, mediated through the production of adenosine.

The failure of theophylline to blunt the vascular response to norepinephrine is further evidence that theophylline does not appear to block the vascular actions of endogenous adenosine. Tissue adenosine content was not increased in either theophylline-treated group as compared with the saline-treated groups, indicating that theophylline did not increase the tissue production of adenosine and override any block that may have been present.

The findings of the adenosine deaminase studies further support

the concept of metabolic regulation of blood flow. The data show that adenosine satisfies one of the conditions that must be met by any proposed vasodilator metabolite; i.e., it must be present in the interstitial space, available to act at the vascular smooth muscle (Berne, 1980). To date, the presence of interstitial adenosine has been inferred from measurements of the total tissue content or the venous outflow of adenosine. The assumption has been made that the amount of adenosine measured was released by the adipocytes and reflected interstitial levels. The interstitial pool of adenosine is that which interacts with the vascular smooth muscle and elicits vasodilation (Berne, 1980). The assumption that tissue or plasma measurements reflect the vasoactive pool has been criticized for failing to account for the existence of an intracellular pool of non-vasoactive adenosine (compartmentalization) (Olsson et al., 1982) or for release of adenosine directly into the blood stream from vascular endothelial cells (bypassing the vascular receptors) (Nees et al., 1983). Therefore, tissue and blood measurements may not accurately reflect the vasoactive pool. In our studies, adenosine deaminase activity was detected in the lymph effluent of the tissue, indicating that the enzyme was present in the interstitial space.

Adenosine deaminase is not believed to reach intracellular spaces. The enzyme is a large protein, approximately 40,000 molecular weight (Gewirtz et al., 1983) and is not expected to penetrate cell membranes. The activity of cytosolic (endogenous) adenosine deaminase is high (Baer et al., 1966), indicating that if the exogenous deaminase was internalized by the adipocytes, the increase in total deaminase activity would be negligible and would not lead to detectable changes

in tissue adenosine content. In our studies, the infusion of adenosine deaminase reduced tissue levels of adenosine by 50%, and the enzyme activity in the interstitial space was inversely related to the tissue adenosine content. Our findings indicate that at least 50% of the tissue content of adenosine was extracellular and available for degradation by the enzyme. The degradation of this pool was associated with a significant increase in vascular resistance under basal conditions, indicating that this pool of adenosine is vasoactive. We also found an inverse correlation between the tissue content of adenosine and vascular resistance, supporting the use of the tissue content of adenosine as an index of the vasoactive pool.

Our data do not support the hypothesis that the vasodilation caused by intravenous norepinephrine is a direct action of norepinephrine on $beta_1$-receptors at the vasculature (Ballard, 1973; Belfrage, 1978a; Rosell and Belfrage, 1979). As we hypothesized, the vascular and the metabolic responses to norepinephrine were dissociated by the removal of adenosine. No vasodilation occurred, yet lipolysis was stimulated, albeit to a lesser degree than seen with norepinephrine alone. In order for a direct action of norepinephrine to account for our findings, one would have to propose that the vasodilation is inhibited because adenosine deaminase somehow interferes with the binding of norepinephrine to the vascular but not to the metabolic receptors. Adenosine deaminase might be proposed to elicit a direct, contractile influence on the vascular smooth muscle that is opposite to the direct, dilatory influence of norepinephrine. However, these proposals are not likely. The specificity of the deaminase treatment supports the hypothesis that the vasodilation was inhibited because endogenous

adenosine was degraded.

Our data indicate that the range of vascular resistances achieved throughout the unstimulated preparations and those in which lipolysis was stimulated is in response to the range of levels of interstitial adenosine. The infusion of adenosine deaminase lowered the tissue adenosine content (Figure 17). In contrast, the intravenous infusion of norepinephrine increased the tissue adenosine content. Over similar time periods, vascular resistance was increased by adenosine deaminase and decreased by norepinephrine (Figure 14). Most of the increased tissue content of adenosine is extracellular, as evidenced by the finding that the combination of deaminase and norepinephrine (ADA/NE) resulted in a level of adenosine that approached that measured in the unstimulated, deaminase-treated group (ADA). The failure of norepinephrine to elicit vasodilation in the presence of adenosine deaminase reflects the decreased level of extracellular (vasoactive) adenosine. The finding that the vascular resistances among the four groups varied inversely with the respective contents of adenosine indicates that vascular resistance is dependent on interstitial adenosine.

We sought to determine whether a substantial enzymatic pathway for the production of adenosine exists in adipose tissue of dogs. We were unable to detect any AMP deaminase activity (degrades AMP to IMP). The levels of 5'-nucleotidase (degrades AMP to adenosine) in control tissues approached those reported for cardiac muscle of dogs and were much greater than those reported for gracilis muscle of cats (Bockman and McKenzie, 1983). In cardiac muscle, adenosine may be a regulator of the circulation (Berne, 1980). In gracilis muscle, other metabolites are proposed to mediate the vascular responses (Bockman and McKenzie,

1983). In cardiac muscle, the ratio of 5'-nucleotidase to AMP deaminase is much greater than that in cat skeletal muscle. The pattern of enzyme activities in adipose tissue is similar to that in cardiac muscle. The relative amounts of these enzymes indicates that as lipolysis is stimulated and tissue levels of AMP increase (Knight and Iliffe, 1973), the potential for the production of adenosine increases.

The removal of endogenous adenosine in unstimulated tissues significantly decreased the glucose uptake, providing the first evidence for a role of adenosine in the regulation of glucose uptake *in vivo*. Although vascular resistance was increased at the same time, the effects of adenosine deaminase on glucose uptake cannot be attributed to a flow-dependent phenomenon. At equivalent blood flow and levels of arterial glucose, the extraction (arterio-venous difference) of glucose was significantly less in the deaminase-treated than in the control animals (data not shown), indicating that the decrease in glucose uptake was a result of the inhibition of cellular uptake. Our hypothesis for the regulation of blood flow focuses on the removal of the intracellular free fatty acids as critical to tissue function. The stimulation of glucose transport would allow an increase in the rate of re-esterification (Vaughan and Steinberg, 1963). Re-esterification, enhanced by glucose uptake, would also limit the accumulation of free fatty acids (Bally *et al.*, 1965; Lisch *et al.*, 1973), and benefit tissue function during metabolic stimulation.

The failure of theophylline to inhibit glucose uptake in unstimulated tissues can indicate at least one of several things. Theophylline may not be an appropriate blocker of the metabolic action of adenosine, as it is ineffective in the blockade of the vascular effects.

The primary action of adenosine to stimulate glucose uptake may be to enhance the sensitivity of insulin (Joost and Steinfelder, 1982; Schwabe et al., 1974; Souness et al., 1983). This action of adenosine may not be affected by the doses of theophylline achieved in our studies. Plasma levels of theophylline measured in three animals were not greater than 50 μM; those reported by Schwabe et al. (1974) to inhibit glucose oxidation were 500 μM to 1 mM. However, as seen in our intraarterial studies, these higher doses of theophylline are vasoactive and therefore would not have been appropriate in our studies. As indicated by the deaminase findings, adenosine clearly modulates glucose uptake in basal, unstimulated adipose tissue.

On the average, adenosine deaminase did not significantly inhibit glucose uptake during the infusion of norepinephrine. If the data from this group (ADA/NE) are considered after deletion of the one animal in which deaminase activity was very low, the data indicate a significant inhibition of glucose uptake by adenosine deaminase in tissues in which lipolysis was stimulated. In this animal, the deaminase activity was insufficient (<0.3 μmol/min/ml) to degrade interstitial adenosine, as evidenced by the high tissue adenosine content. Thus, the level of glucose uptake is dependent on the levels of interstitial adenosine. If one assumes a release rate of adenosine by basal adipocytes to be 0.1 nmole/min/g (Fredholm and Sollevi, 1981) and the interstitial volume to be 10% of the tissue weight (Linde and Chisholm, 1975), with a measured deaminase activity of 0.4 μmoles/min/ml interstitial fluid, the interstitial concentration of adenosine can be estimated with the Michaelis-Menten velocity equation. In unstimulated tissue, the interstitial concentration of adenosine is estimated at 0.1 μM. Norepinephrine

increases the release of adenosine as much as ten-fold (Fredholm and Sollevi, 1981). At this increased rate of release, given a similar activity of deaminase in the interstitial space, the interstitial concentration of adenosine would be estimated at 1.0 μM. The levels of interstitial adenosine achieved after infusion of adenosine deaminase in the stimulated group (ADA/NE) could vary as much as ten times those in the unstimulated group (ADA). These variable levels of adenosine could account for the diminished effect of the deaminase on glucose uptake in the stimulated group as compared with the unstimulated group.

Our results indicate a significant inhibition by theophylline of the glucose uptake elicited by norepinephrine. Although an inhibition by theophylline is in keeping with the hypothesis of a role of adenosine, caution should be exercised in attributing this finding to antagonism of adenosine. Theophylline did not antagonize other actions of adenosine so this effect may not be related to adenosine. If this finding is indicative of blockade of adenosine receptors, a difference in the sensitivity of the metabolic (glucose) receptors and the vascular receptors would be apparent. The effect of theophylline may also be related to another aspect of adipose tissue metabolism. Although the plasma concentration of insulin among the theophylline groups was not measured, it can be assumed that the plasma levels of insulin would be higher in the glucose-infused, norepinephrine groups than in the unstimulated groups. Theophylline has been shown to decrease insulin binding (Joost and Steinfelder, 1983), and this action may be responsible for the decrease in glucose uptake observed in the group that received theophylline and norepinephrine. The finding that theophylline failed to block the vascular effects of adenosine detracts from a conclusion

that the effects of theophylline on glucose uptake are related to adenosine. The mechanism for the effect may be related to an undefined influence of theophylline on metabolism.

We did not find an increase in the basal lipolytic rate, as measured by glycerol release, with either adenosine deaminase or theophylline. In our preparations, the release of glycerol in unstimulated tissues was low, often at the lower limit of detection. Therefore, small but significant increases in glycerol release may not have been detected. The finding that adenosine deaminase did not increase this basal release of glycerol is in opposition to many in vitro studies in which adenosine deaminase significantly increased the basal lipolytic rate (Dietrich and Schwabe, 1975; Ohisalo, 1981; Shechter, 1982; Turpin et al., 1977). In in vitro studies, the isolated adipocytes are bathed with a medium containing high amounts of albumin which serve as a trap for the released free fatty acids. The medium usually contains high amounts of glucose, as well. Both these factors would favor an increased lipolytic rate, by providing a protein carrier and by increasing the rate of re-esterification, respectively (Bally et al., 1965; Bulow and Madsen, 1981; Chlouverakis, 1967; Knight and Iliffe, 1973; Rodbell, 1965; Scow et al., 1965). In our studies, the infusion of adenosine deaminase significantly increased vascular resistance, perhaps reducing the delivery of albumin so that the diffusion of free fatty acids was impaired. Adenosine deaminase also significantly reduced the glucose uptake. A stimulation of lipolysis by adenosine deaminase would not be evident if the lack of protein carrier and a decreased rate of re-esterification promoted an accumulation of free fatty acids. This accumulation would inhibit the lipolytic rate. Our data indicate a tendency for glycerol

release to be decreased by adenosine deaminase, perhaps due to the concommitant effects of the deaminase on vascular resistance and glucose uptake.

The inability of theophylline to stimulate the basal lipolytic rate indicates that significant changes in glycerol release were not detected, that theophylline does not antagonize this proposed action of adenosine, or that adenosine has no role in the regulation of lipolysis in unstimulated tissues. The failure of theophylline to increase the basal lipolytic rate *in vivo* supports findings of Sollevi *et al.* (1981). Theophylline (30-60μM) did not enhance basal glycerol outflow in their studies. However, in our studies, theophylline does not appear to antagonize the effects of exogenous or endogenous adenosine. In addition, specific degradation of adenosine with adenosine deaminase did not increase the basal lipolytic rate. Our data do not support a role of adenosine in the inhibition of lipolysis nor the use of theophylline as an antagonist of adenosine. Given these observations, failure of theophylline to stimulate the basal lipolytic rate is not surprising.

Adenosine does not appear to inhibit catecholamine-stimulated lipolysis. The lipolytic response to intravenous norepinephrine was not potentiated by the infusion of adenosine deaminase or theophylline. Again, a tendency for the glycerol release to be less in deaminase-treated (ADA/NE) than in control (NE) was observed. Our findings support those of Shechter (1982) in which adenosine deaminase failed to enhance catecholamine-stimulated lipolysis in strips of adipose tissue. In contrast, Shechter did observe enhanced lipolysis with adenosine deaminase in isolated adipocytes. Because Shechter proposes that the *in vivo* situation is more accurately represented in intact strips rather

than in isolated adipocytes, he concluded that adenosine has no role in the inhibition of hormone-stimulated lipolysis in vivo. However, in his studies, adenosine deaminase may not have reached the interstitial spaces of his strips, possibly invalidating any conclusions about the role of adenosine. In our preparations, adenosine deaminase was measured in the lymph fluid and no enhancement of glycerol release was observed. At the same time, adenosine deaminase increased vascular resistance and decreased glucose uptake. Although it does not appear that adenosine inhibits hormone-stimulated lipolysis, such a role of adenosine cannot be ruled out, because blood flow and metabolism were also affected.

The results of our studies with theophylline also emphasize the dynamic balance between re-esterification and lipolysis (Steinberg and Vaughan, 1963). When the glucose release was significantly inhibited by theophylline, a significant decrease in the lipolytic rate was observed. In contrast to our studies, Sollevi et al. (1981) showed theophylline to enhance the lipolytic response to nerve stimulation. The discrepancies between our findings and those of Sollevi et al. (1981) may reflect the different experimental conditions. We studied the effects of theophylline on the lipolytic response to intravenous norepinephrine. During administration of norepinephrine, blood flow does not decrease significantly and no hypoxia ensues. During stimulation of the sympathetic nerves, a severe vasoconstriction occurs. The metabolic events that accompany this vascular response are expected to be quite different from those that occur with the intravenous infusion of norepinephrine.

In two animals that received adenosine deaminase and norepinephrine (ADA/NE), an apparent stimulation of glycerol release was observed.

However, an influence of adenosine on lipolysis could possibly be attributed to the response in only one animal. Tissue adenosine in this animal was very low, indicating that the decrease in interstitial levels of adenosine may have been sufficient to stimulate lipolysis. Graded decreases in tissue adenosine content were observed in the group that received adenosine deaminase and norepinephrine. Yet, a parallel, graded stimulation of lipolysis was not observed. Therefore, it is unlikely that metabolic response in this one animal reflects a role of adenosine in the inhibtion of lipolysis. In the second animal, the interstitial deaminase levels were less than 0.3 μmoles/min/ml, and the tissue adenosine content was very high. The lipolytic response in this second animal was more like that in the norepinephrine control group than that in the group that received adenosine deaminase and norepinephrine. Consistent evidence for a role of adenosine in the inhibition of lipolysis was not found in these studies. If any feedback by adenosine exists, it appears to be overridden by the other actions of adenosine on metabolic and vascular events.

Our studies support the concept of metabolic regulation of blood flow in adipose tissue of dogs. The infusion of an enzyme to degrade adenosine specifically increased vascular resistance, evidence for a cause-and-effect relationship. Our data provide evidence that a large vasoactive (extracellular) pool of adenosine exists in adipose tissue, approximately 50% of the total tissue content, and that changes in this pool are reflected by our measurements of total tissue content. We have shown that a substantial enzymatic route for the production of adenosine exists, indirectly supporting the metabolic hypothesis. Lipolysis, induced by intravenous norepinephrine, is closely associated

with the vasodilation and appears to be linked, at least in part, by the production of adenosine. Adenosine also appears to stimulate glucose uptake *in vivo*. The removal of endogenous adenosine decreases glucose uptake and blood flow and these events may, in turn, affect the overall metabolic rate at adipose tissue. Vasodilation and glucose uptake appear to be important to the continuation of lipolysis. Our findings support the hypothesis that the metabolism of adipose tissue directs the blood flow, and that adenosine may be the major mediator of the vascular events.

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