Adrenergic Neurohumoral Influences on Circulation and Lipolysis in Canine Omental Adipose Tissue

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ABSTRACT

Vascular resistance, capillary filtration coefficient (CFC) and changes in blood volume were determined in canine omental adipose tissue. Release of glycerol and free fatty acids (FFA) were measured. Basal values in acutely denervated tissue for blood flow and CFC were 11 ml/min/100 g (range 4 to 26, n = 38) and 0.05 ml/min/100 g/mm Hg (range 0.018 to 0.090, n = 38), respectively. Stimulation of the sympathetic nerves (1 to 9 Hz) caused initial vasoconstriction, maintained for 1 to 3 minutes. At lower frequencies (1 to 3 Hz), the blood flow increased gradually after the initial decrease. At higher frequencies, the constriction often reverted to vasodilatation. The CFC was not changed or increased initially. The clearance rate of locally injected I\(^{125}\)I decreased in spite of a constant blood flow and blood volume was reduced initially. After \(\alpha\)-receptor blockade, nerve stimulation caused vasodilatation, which in turn was inhibited by \(\beta\)-receptor blockade. Infusion of norepinephrine produced a pattern of vascular and lipolytic responses similar to those evoked by nerve stimulation and by norepinephrine. The lipolytic response was inhibited by \(\beta\)-receptor blockade. It is concluded that sympathetic nerves are physiologically important for the regulation of vascular reactions and control of lipid metabolism in omental adipose tissue. Whether this is also the case for circulating catecholamines remains to be established.

KEY WORDS: capillary filtration coefficient, sympathetic nerves, norepinephrine, blood flow, glycerol, \(\alpha\)- and \(\beta\)-receptor blockade.

Observations on adipose tissue in vitro indicate that there are quantitative differences in the control of the lipid metabolism in tissues from different localities. Wertheimer et al. (1) found that mesenteric adipose tissue from the rat was more sensitive to epinephrine than perirenal or epididymal tissue. Regional differences in the response to norepinephrine have also been found in human adipose tissue. For example, it produced a stronger lipolytic effect in omental than in subcutaneous tissue in vitro (2). From recent studies it became evident that characteristic differences in vascular as well as metabolic responses to adrenergic neurohumoral activity can occur. The canine mesenteric vascular bed responded much more weakly to sympathetic nerve
activity than did the subcutaneous vessels (3). Furthermore, stimulation of sympathetic nerves failed to increase lipolysis in canine mesenteric fat in contrast to the powerful lipolytic effect in subcutaneous adipose tissue (8). It was postulated that the regional differences in the capacity of sympathetic nerves to influence lipolysis result from differences in the distribution and the activity of sympathetic nerve terminals. These differences were explained on the basis that a greater concentration of sympathetic nerve terminals is present in mesenteric fat compared to subcutaneous fat (4). This explanation was not supported by anatomical studies, since sympathetic nerves failed to increase lipolysis in canine omental fat (3.

In all experiments except those in which capillary filtration coefficient (CFC) and changes in regional blood volume were determined, the tissue was perfused with a plethysmograph filled with Tyrode's solution at 38°C (8, 9). The tissue was perfused from the femoral artery, and the venous outflow was allowed to drip freely into an open funnel provided by an apparatus connected to a reservoir to measure blood flow. The time between heparin administration and the experiment was considered to allow the activity of the CFC the venous pressure was elevated for 2 to 3 minutes by raising the free end of the outflow cannula to a height of 10 cm. It was assumed that 4/5 of the rise of the venous outflow pressure was transmitted to the exchange vessels (9). The slow gain in tissue volume due to filtration at elevated venous pressure was used to calculate CFC, which is expressed as ml/min/100 g/mm Hg. Changes in regional blood volume at nerve stimulation were calculated from the initial rapid decrease in tissue volume as blood was expelled from the capacitance vessels (8). The cut ends of the perivascular nerves to the isolated section were stimulated with square pulses by bipolar silver electrodes activated by a Grass stimulator. Frequencies from 1 to 9 Hz were applied, with a duration of 2 msec and an intensity of 8 to 10 v. Stimulation was applied for time intervals of 5 to 30 minutes.

The washout of 125I was measured from a local injection site according to Kety (10), in tissue perfused at constant flow. The 125I, stabilized with sodium thiosulfate, was dissolved in isotonic saline solution (Studsvik, Sweden). Threeiliters physiological saline was injected. The isotope was monitored with a scintillation detector (Philips PW 4125). A preamplifier and pulse height analyzer fed into a scaler connected to a digital printer. Physical background was usually 500 counts/min.

A fresh solution of L-norepinephrine bitartrate (Astra, Sweden), was made for each infusion and the infusion syringe was encased in an ice bag throughout the experiment. The solution was infused at a constant rate (0.025 ml/min) via a sidearm in the arterial cannula. The concentrations are expressed as equivalent amounts of base. Propranolol (Inderal), a /3-receptor blocking agent, and phentolamine (Regitin), a-receptor blocking agents, were injected intrarterially. A different solution of L-norepinephrine bitartrate (Astra, Sweden), was used for each infusion and the infusion syringe was encased in an ice bag throughout the experiment. The solution was infused at a constant rate (0.025 ml/min) via a sidearm in the arterial cannula. The concentrations are expressed as equivalent amounts of base. Propranolol (Inderal), a /3-receptor blocking agent, and phentolamine (Regitin), a-receptor blocking agents, were injected intrarterially. Samples of arterial and venous blood (1 to 2 ml) were collected simultaneously at intervals in centrifuge tubes placed in ice, and the plasma was analyzed for glycerol (11) and free fatty acids (FFA) (12). In 100 duplicate determinations, the standard deviation for FFA and glycerol was ± 0.05 and ± 0.03, respectively. The heparin was determined in several arterial samples. The net release, or uptake, was determined from the AV concentration difference times plasma flow. Resistance in the vascular bed is expressed in peripheral resistance units, i.e., blood pressure/blood flow. To obtain resting values of blood flow, CFC, glycerol, and FFA
release, three determinations were made during the first 30 minutes of the experimental run before any stimulations or infusions were started.

Blood flow and systemic arterial pressure were recorded continuously on a Grass polygraph. The tissue volume was recorded on a smoked kymograph drum.

**Results**

**Vascular and Lipoytic Characteristics of Omental Adipose Tissue**

The resting blood flow of omental adipose tissue averaged 11 ml/min/100 g (range = 4 to 26 ml/min/100 g, N = 30). It could be increased to 40 to 45 ml/min/100 g with a potent dilating agent (theophylline, 15μ).

CFC during the resting state was measured in 12 dogs (36 determinations). The mean value was 0.050 ml/min/100 g/mm Hg (range = 0.018 to 0.090).

Glycerol was determined in 17 animals and the mean net release during rest was $0.22 \pm 0.10 \mu$moles/min/100 g ($N = 49$), whereas there was a net uptake of FFA ($0.57 \pm 0.24, N = 17, 6$ animals).

**Responses to Nerve Stimulation**

**Vascular Reactions.**—The nerves to the omental tissue were stimulated in 19 dogs (49 stimulations) at frequencies ranging from 1 to 9 Hz. Initial constriction occurred at all frequencies. When stimulation was applied continuously for 15 to 30 minutes, a pattern of response emerged which differed according to the frequency of impulses. At 1 to 3 Hz, blood flow was reduced initially; then, in most experiments, it was gradually increased but did not reach resting levels. In some cases a compensatory hyperemia followed termination of the stimulation. At higher frequency (4 to 6 Hz), illustrated in Figure 1, middle section, the initial constriction was maintained for 1 to 6 minutes, then the flow increased to the resting level for the remainder of the period of stimulation. Compensatory increase in flow lasted for 1 to 4 minutes at the termination of stimulation. A third pattern of response was observed at a frequency of 6 to 9 Hz. The initial constriction prevailed for 1 to 3 minutes. However, in this case the flow increased rather rapidly to above resting level and remained there for the rest of the stimulation period. Poststimulation hyperemia was observed in these experiments also.

Responses of the blood flow to stimulation at different frequencies are shown in the series of topmost curves in Figure 2. After treatment with dihydroergotamine ($N = 3$), 100μg ia, or phentolamine ($N = 2$), 300 μg ia, nerve stimulation caused vasodilatation only. This in turn was inhibited by propranolol, 100 μg.

Figure 2 also shows the mean values of the CFC in the omentum during nerve stimula-
Mean values and se for blood flow, capillary filtration coefficient (CFC), and change of tissue volume (11 dogs) during electrical stimulation of the nerves with different frequencies (1/s, N = 15; 3/s, N = 34; 4/s, N = 15; 6/s, N = 17; 9/s, N = 20). The open symbols indicate basal values. The solid symbols indicate three consecutive values during the stimulation period (15 to 30 minutes).

Increased CFC indicates facilitated hydrodynamic conductivity of the vascular bed due, for example, to an alteration in permeability or in the size of the surface area available for exchange. In an attempt to differentiate between these two possibilities, the clearance of locally injected 125I was studied in seven dogs. Since the clearance rate is also influenced by changes in total blood flow, a technique for perfusion at constant flow was employed. At frequencies of 3, 6 and 9 Hz, the clearance rate was reduced in 14 out of 15 stimulation periods. A representative experiment is shown in Figure 3. It is seen that the clearance rate was reduced during the whole stimulation period. In some experiments an initial reduction was followed by a gradual return of the clearance rate during the latter part of the stimulation period. However, the clearance rate during nerve stimulation never exceeded the resting rate. 

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Peripheral resistance, release of free fatty acids, and glycerol in canine omental adipose tissue. Tissue weight 25 g. Electrical stimulation of the nervous supply (1 Hz and 16 Hz respectively, 10 v, 2 msec). Autoperfusion. Resting blood flow 6.5 ml/min/100 g. PRU = peripheral resistance units.

Capacitance vessels responded immediately during nerve stimulation. This was evident from the initial rapid decrease in tissue volume as blood was expelled from the venous system. As shown in Figure 2, when stimulation was applied, the tissue volume was quickly reduced at all frequencies employed, then gradually returned toward prestimulatory levels.

Lipolytic Responses. The outflow of glycerol and FFA from omental adipose tissue (n = 14) was increased during nerve stimulation when the frequency was 1 Hz or more (Figs. 1 and 4). The lipolytic responses were eliminated by propranolol (100 μg) but not by an α-receptor blocking agent (dihydroergotamine, 100 μg).

INFUSION OF NOREPIPEPHRINE
Norepinephrine was infused continuously for 15 to 30 minutes (5 dogs) in amounts to obtain plasma levels of added catecholamines from 0.001 to 0.05 μg/ml. It produced changes in total blood flow that closely resembled the effect of sympathetic nerve stimulation. Figure 5 is a record taken during infusion of norepinephrine to give a concentration of added catecholamine equal to 0.05 μg/ml plasma. The similarity between effects of norepinephrine and nerve stimulation on vascular and lipolytic responses in omental fat can be seen by comparing this figure with Figure 1. Glycerol output increased when the added catecholamine reached 0.05 μg/ml (Fig. 5). However, the number of experiments at lower concentrations was too small to establish the threshold concentration.

Discussion
It is reasonable to assume that the observed effects of electric nerve stimulation were due
to activation of adrenergic sympathetic nerve fibers. In favor of this assumption are the facts that the vasoconstriction was blocked by adrenergic α-receptor blocking agents whereas the vasodilatation and the lipolytic responses were inhibited by adrenergic β-receptor blockade. This resembles the pattern of blockade observed in canine subcutaneous adipose tissue (13). Furthermore, the vasoconstriction and the lipolytic effects could be mimicked by infusion of norepinephrine.

In earlier studies, the influence of the adrenergic neurohumoral system on lipolysis and blood flow was examined in mesenteric (3) and subcutaneous adipose tissue of the dog (4, 5, 13-17). Those studies and the present one show that there are regional differences in the regulation of lipolysis and blood flow in adipose tissue by the adrenergic neurohumoral system which may be great enough to be of physiological importance. For example, norepinephrine induced lipolysis in all three tissues, but the omentum and the subcutaneous adipose tissue appear to be much more sensitive than the mesentery. Lipolysis in mesenteric adipose tissue required such large amounts of added norepinephrine that physiological concentrations presumably were exceeded. Furthermore, the mesentery did not respond with enhanced lipolysis to sympathetic nerve stimulation, in contrast to subcutaneous and omental tissue.

The present finding that lipolysis is already enhanced at a stimulation frequency of 1 Hz indicates that the sympathetic nerves to the omental tissue are of importance in the regulation of lipolysis. This conclusion is based on the observation that the impulse frequency in sympathetic nerves, at least to skeletal muscle, is 3 to 2 Hz during resting conditions and may increase to 8 to 10 Hz during maximal activity (18). Recent studies indicated that with respect to lipolysis the sympathetic innervation of subcutaneous adipose tissue is of greater importance than circulating catecholamines (5). Whether the concentration of circulating catecholamines in intact animals is high enough to induce lipolysis in omental tissue remains to be evaluated.

Quantitatively, resting blood flow in omental adipose tissue (11 ml/min/100 g) is within the same range as in the mesenteric (10 ml/min/100 g) (3) and subcutaneous adipose tissue (8 ml/min/100 g) (19), and the maximal blood flow obtained is the same in subcutaneous and omental tissue (16). Vasoconstriction following sympathetic nerve stimulation is seen in all three tissues. However, the vascular bed of the mesentery responds very weakly to frequencies within the physiological range (3), and the present experiments showed that the initial vasoconstriction is not maintained in the omentum to the same extent as it is in subcutaneous tissue. In fact, in spite of continued stimulation, the constriction at frequencies above 6 Hz often reverted to vasodilatation. A similar reaction was observed during infusion of norepinephrine. The attenuation of the constriction in omental tissue may be of significance under certain pathophysiological conditions. Kovách et al. (20) have shown that during severe hemorrhage, the vasoconstriction in canine subcutaneous adipose tissue is stronger than in most other organs. Irreversible vascular damage seemed to occur, since the blood flow could not be restored by infusion of the shed blood. If, as in the omental tissue, the vascular bed could overcome the restricted blood flow in spite of continued intense adrenergic activity and thereby maintain a fairly normal tissue metabolism, damage would be less likely to occur. It would be of interest, therefore, to study the response to hemorrhage in the omentum.

Resting capillary filtration coefficient (CFC) in omental adipose tissue was found to be approximately twice as high as in subcutaneous adipose tissue (16) and about three times as high as in resting skeletal muscle (9). This shows that when there is an increased venous pressure the capacity for filtration is greater in the omentum than in the other two tissues. There may be several explanations for this difference. More capillaries per unit weight may be patent, perhaps...
as a consequence of higher metabolism. Alternatively, there may be a greater total number of capillaries per unit weight in omental adipose tissue or they are more permeable. It is interesting to note that Zweifach and Intaglietta (21) have demonstrated that single capillaries in the rabbit cecum are more permeable than those of skeletal muscle.

A conspicuous characteristic of subcutaneous adipose tissue circulation is that vasoconstriction is accompanied by an augmented CFC when the sympathetic nerves are stimulated. The reason for this is not clear, but the possibility of an increased permeability has been considered (15, 17). Under similar experimental conditions, CFC in the cecum increased or remained unchanged. However, the initial augmentation was not as marked as in subcutaneous tissue, where CFC could be tripled. Furthermore, with continued stimulation an initially increased CFC response in the omentum gradually diminished (Fig. 2). The exchange of $^{125}$I was decreased during nerve stimulation. Since the perfusion flow was kept constant, this would indicate a reduced area for solute exchange. A similar reaction has been noticed in subcutaneous adipose tissue (Linde and Rosell, unpublished). One would expect a reduced CFC under these circumstances, but this was not the case. The reason may be a concomitant increase in permeability, as was mentioned above. However, we present here no direct experimental evidence to support this hypothesis.

References

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