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 125I decreased in spite of a constant blood flow and blood volume was reduced initially. After α-receptor blockade, nerve stimulation caused vasodilatation, which in turn was inhibited by β-receptor blockade. Infusion of norepinephrine produced a pattern of vascular and lipolytic responses similar to those evoked by nerve stimulation. The release of glycerol and FFA was increased by nerve stimulation and by norepinephrine. The lipolytic response was inhibited by β-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 resistance capacitance free fatty acids blood flow glycerol α- and β-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 (3). The difference between the two may be related to the presence of autoregulatory mechanisms in subcutaneous fat that were lacking in mesenteric fat. Experimental data from portal-splanchnic flow studies in dogs (2, 3) and human subjects (4) have shown that adipose tissue in the gut wall has a lower metabolic rate than that of the subcutaneous fat. This difference may be due in part to local differences in the supply of nutrients and oxygen and to the presence of a larger concentration of nonadipose tissue in the gut wall. However, the most important factor may be a difference in the properties of the adipose tissue itself. Adipose tissue from different regions of the body may be able to respond differentially to sympathetic nerve stimulation. For example, it has been shown that sympathetic stimulation of the splanchnic nerves increases lipolysis in canine mesenteric adipose tissue (3) but not in subcutaneous adipose tissue (3). This difference may be due to differences in the extent of sympathetic innervation of the two tissues. Sympathetic nerves to mesenteric adipose tissue are more densely packed than those to subcutaneous adipose tissue (5). Therefore, it is possible that the difference in lipolytic response is due to differences in the number of sympathetic nerve fibers innervating the two tissues.

Methods

Omental adipose tissue was isolated in 37 mongrel dogs of both sexes, anesthetized with sodium pentobarbital (30 mg/kg, iv) with supplement as necessary. The procedure was as follows. The spleen was exteriorized to provide traction and preserve anatomical integrity of the greater curvature of the stomach. A branch of the splenic artery, the vein, and the nerve bundles accompanying the vessels were dissected free at the level of the greater curvature of the stomach. A portion of the omental tissue supplied by these structures was separated by ligatures from the remainder of the omental sheet. Finally, the spleen was removed. Wet weight of the omental tissue obtained at the end of the experiment averaged 39 ± 1.8 g (se). About 30 to 60 minutes after administration of heparin (15,000 IU, iv), autoperfusion (30 dogs) of the isolated section was provided via a cannula in the femoral artery, interrupted by a drop chamber to measure blood flow. The time between heparin administration and the experiment was considered to allow the activity of the clearing factor lipase to reach a steady level (5, 6). In seven dogs a constant flow perfusion was provided by an apparatus connected to a reservoir containing the animal's own blood (7). The venous outflow was directed into the femoral vein and the nerve bundles accompanying the vessels were dissected free at the level of the greater curvature of the stomach. A portion of the omental tissue supplied by these structures was separated by ligatures from the remainder of the omental sheet. Finally, the spleen was removed. Wet weight of the omental tissue obtained at the end of the experiment averaged 39 ± 1.8 g (se).

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INFLUENCES ON OMENTAL ADIPOSE TISSUE

RESULTS

VASCULAR AND LIPOLYTIC 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, 15µM). CFC during the resting state was measured in 12 dogs (38 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 ± 0.10 µmoles/min/100 g (N = 49), whereas there was a net uptake of FFA (0.57 ± 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 i.a., or phentolamine (N = 2), 300 µg i.a., 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 = 24; 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).

Mean CFC was always increased in experiments in which a change was observed. However, the augmented CFC was seen during the initial part of the stimulation period only. Thereafter the CFC returned to the prestimulatory level.

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 $^{131}$I 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.
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 NOREpinephrine

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 1 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. Kováč 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 omentum 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 omentum 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 125I 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, at present we have no direct experimental evidence to support this hypothesis.

References


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