CORTICOSTERONE MODULATES THE VASODILATION BY PERIVASCULAR FAT

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Abstract

Adipose tissue is presently considered to be an active endocrine and paracrine organ. Obesity is closely related with several disorders including hypertension. Obese seem to have a dysfunctional adipose tissue, which shows an ongoing inflammation, and the production of cytokines regulating inflammation as well as more specific adipokines are changed. Also an elevated activity of 11β-HSD-1 is seen in these patients. Perivascular fat has been shown to attenuate vascular responsiveness. Our study aims to investigate if inflammation, specifically corticosterone, in adipose tissue changes the vasodilatory effect of perivascular fat.

Mesenteric small arteries from adult, female, Sprague-Dawley rats were isolated and mounted in a Mulvany-Halpern myograph. Perivascular fat was initially removed from all vessels and their noradrenaline sensitivity determined. Next perivascular fat was wrapped around every other vessel followed by a new determination of their noradrenaline sensitivity. Finally the noradrenaline sensitivity was determined after previous addition of corticosterone (10 μM).

In accordance with previous reports we found that addition of fat around the vessels caused a significant reduction in noradrenaline sensitivity (Δlog EC50 -0,369± 0.083, n=10) and a reduction in maximal response (-10,7%± 2,45). Addition of corticosterone partially anatagonised the vasodilatory effect of perivascular fat. The noradrenaline sensitivity tended to increase (Δlog EC50 0.182± 0.110, n=8) in vessels with fat and corticosterone compared with vessels with fat, but no corticosterone present. There was no effect on vascular responsivness in vessels without perivascular fat before and after addition of corticosterone.

These data suggest that corticosterone may modulate the vasodilatory effect of perivascular fat.

Populärvetenskaplig sammanfattning

Nytt hopp för överviktiga

Övervikt kan förkorta liv dramatiskt. En del av problemet hos överviktiga är det höga blodtrycket. Tills nyligen har sambandet mellan vikt och blodtryck varit ett mysterium. Men nu börjar forskare ana ett samband mellan fettväv och blodtryck.

Kroppens fettceller spelar en avgörande roll i kontrollen av blodtrycket. Detta har forskare kunnat konstatera när de gjort prover på hur blodkärl påverkas av omgivande fettväv. När mycket fettväv samlas runt ett blodkärl behövs högre doser av ett kärlsammandragande ämne för att kärlet ska kunna fungera optimalt.

När man undersökt fettceller hos en grupp överviktiga och jämfört dem med fettceller hos normalviktiga, har det visat sig att hormonet Cortisol är förhöjt i de överviktigas celler. Cortisol är ett ämne som påverkar inflammatoriska tillstånd. Att överviktiga också ofta har inflammation i fettväven är därför inte förvånande. Men det slutar inte där: inflammationstillståndet i fettväven är troligen orsaken till en förändrad utsöndring av de ämnen som normalt kommer från fettceller. Forskare misstänker nu att de ämnen som utsöndras påverkar hur blodkärlen dras samman.

Eftersom såväl övervikt som högt blodtryck är ökande hälsoproblem i västvärlden är resultatet av forskningen viktig. Om man i framtiden kunde få klarhet i hur ämnen som utsöndras från fettceller påverkar andra celler i kroppen, skulle detta kunna leda till mycket bättre och effektivare behandlingsmetoder för den stora grupp patienter som lider av övervikt och högt blodtryck. Kan man komma åt ämnen som påverkar blodkärlens sammandragning på cellnivå finns hopp om att många liv kan räddas.

Introduction

Practically all blood vessels are enclosed in adipose tissue(1). Perivascular adipose tissue are these days not only considered to be a tissue mainly for energy storage, support and insulation, but an active endocrine and paracrine organ as well. Several studies have demonstrated that perivascular fat attenuates vascular responsiveness. Soltis and Cassis were the first to demonstrate that perivascular fat significantly attenuates the vascular responses to noradrenaline of aortic ring preparations from rats in vitro(2). Their suggestion was that uptake of noradrenaline by the surrounding adipose tissue was the reason for the observed anticontractile effect. Others have further looked into the matter and have instead suggested that one or several different factors released from the perivascular tissue are responsible for the vasodilatory effect. Suggested substances have been leptin(3), adiponectin(4)(5) and ADRF(4)(6) (adventitiumderived relaxing factor). Finding out if one or several of these factors play a part in regulating blood pressure and what in turn regulates the production and secretion of them could bring us closer to an understanding of the development of hypertension.

Obesity is today a growing health problem closely associated with the metabolic syndrome, a combination of disorders including insulin resistance, diabetes, dyslipidemia, and hypertension(7)(8)(9). Obesity is characterized by adipose tissue inflammation and a change in production of proinflammatory cytokines as well as different more specific adipocytokines(10). Among these is a well documented reduced plasma concentration of adiponectin(11). The inflammation seen in obesity seems to lead to a dysfunction in the release of adipocytokines. A lower concentration of at least one, but maybe several, potential vasodilators might explain the hypertension associated with obesity.

In obese humans, adipose tissue has elevated activity of 11β-HSD-1 (12), an enzyme that converts the biologically inactive cortisone to active cortisol (13). The plasma concentration of cortisol in obese seems to be normal, but the intracellular concentration is high (14). Glucocorticoids have been shown to decrease the levels of

adiponectin in animal models (15) and humans (16) and inhibition of 11β-HSD-1 has been shown to increase adiponectin concentration in mice (7). Levels of leptin are raised where the 11β-HSD-1 activity is high(17) as well as in obesity(18)(19). The change in enzyme activity followed by higher intracellular concentrations of cortisol might explain the observed changes in circulating adiponectin and leptin seen in obesity. A change in production and/or secretion of one or several vasodilators originating from adipose tissue could be an explanation for hypertension associated with obesity.

Since fat has been suggested to be secrete potent vasodilator, and its production and/or secretion seem to be connected with cortisol, we wanted to further investigate the vasodilatory effect of perivascular fat on small vessels and how that vasodilatation is affected when cortisol is present.

Materials and methods

Methods

Female Sprague-Dawley rats (270-360 g, 8-11 weeks old from a local colony, n=9) were used, The rats were killed with CO_2 inhalation and the intestine was removed and instantly placed in cold PSS (Physiological Salt Solution) (119 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4*7H_2O$, 25 mM $NaHCO_3$, 1.6 mM CaCl2*$2H_2O$, 0.026 mM EDTA, 5.5 mM glucose), oxygenated with 95% O_2/ 5% CO_2. The first or second branches from the superior mesenteric artery were dissected into 2-mm rings. Perivascular fat was removed carefully using scissors so as not to damage the adventitia. Pieces of 80-110 mg (excess liquid absorbed on paper prior to weighing) of perivascular fat were separated and stored for later separate mounting in the myograph. All animal procedures were in accordance with national guidelines for animal research.

Isometric contractions

The arterial segments were mounted in a wire myograph (Danish Myo Technology model 610M) onto two 40 µm wires carefully inserted through them. One of the wires was connected to a force transducer and isometric tension was recorded and displayed on a computer. The myograph organ bath contained 5 ml PSS.

The organ baths were continuously oxygenated with 95% O_2/ 5% CO_2 to provide oxygen and to maintain pH 7.4. Temperature was kept at 37ºC. The vessel wall tension and diameter were normalized after a 30 minutes stabilization period using a standardized procedure(20).

The vessel rings were set to a resting tension corresponding to 0.9 of the internal diameter the vessels would have at a transmural pressure 100mmHg if rounded. The vessels were maximally activated by changing the bath solution to 5 ml K+-PSS (123.7 mM KCl, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4*7H_2O$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2*2H_2O$, 0.026 mM EDTA, 5.5 mM glucose (95% O_2/ 5% CO_2)) and 10 µM noradrenaline for 2 minutes for the vessels to contract, the vessels were then given a 5 minutes rest in PSS, and the procedure was performed a total of three times). The third contraction was used to establish a baseline for maximal contraction(20).

Concentration response curves to noradrenaline over the range 10^{-8} to $4*10^{-5}$ mol/L were performed, doubling the concentration every three minutes. The first curve was determined where all vessels were without fat, ((-)fat vessels), followed by one curve were fat was put around every other vessel, ((+)fat) vessels. Before a third curve 10 µM corticosterone was added to the bath 10 minutes before the addition of noradrenaline. The experiment were completed within 8 hours of killing the rat.

At the end of the experiment the vessels were again maximally activated with K+-PSS and noradrenaline to ensure that they had not changed their contractile properties during the experiment.

All drugs were obtained from Sigma.

All values are given as mean ±SEM. Statistics was by means of Students t test with a P-values < 0.05 regarded as significant. N represents the number of individuals tested. In most experiments four vessels were used, two (-)fat and two(+)fat. [NA] represents concentration of noradrenaline.

Results

A different technique than in previous reports on the effect of perivascular fat on a vessel's contractile response to different vasoconstrictors was used(21). First all perivascular fat was removed around the vessels and a concentration response curve to noradrenaline was performed. Before repeating the cumulative response curve, fat was wrapped around every other vessel. To avoid changes in contraction response due to the possibility that fat could work as a diffusion barrier, the fat was wrapped making sure to leave some space over the vessel. Using this technique it can be observed how the same vessel changes its contractile response when exposed to perivascular fat in its close proximity compared to when the vessel had no exposure to fat. We could determine that vessels with perivascular fat showed a significant reduction in noradrenaline sensitivity (Δlog EC50 -0,369± 0.083, n=10) (Fig. 1 and Fig. 2) and a reduction in maximal response (-10,7%± 2,45) (Fig. 1 and Fig. 3). In control vessels, where no fat was added, the first two curves showed similar response (n=10)(Fig. 4).

Next the effect of corticosterone on the observed effect of perivascular fat on vasoconstriction was examined. When exposed to 10 µM corticosterone, the sensitivity to noradrenaline tended to increase in the vessels with fat wrapped around (Δlog EC50 0.182± 0.110, n=8) (Fig. 1 and Fig. 2) , partially reversing the effect of perivascular fat. The maximal response was not affected. Corticosterone also appeared to have a lowering effect on the maximal response of vessels without perivascular fat (Fig. 3 and Fig. 4).

Discussion

Two hypoteses were tested in this study. First, it was hypothesized that perivascular fat has an inhibiting effect on a vessel's contractile response to noradrenaline. Secondly, the hypothesis was tested that the presence of cortisol would diminish the observed effect of perivascular fat.

Our data implies that perivascular fat indeed is involved in the regulation of vascular tone in mesenteric arteries. This study demonstrates findings similar to those obtained in previous studies (21).

Soltis and Cassis found an attenuated vascular responsiveness to noradrenaline of intact aortic ring preparations from rats compared to vessels where periadventitial fat was removed(2). They could eliminate the effect by administering the neuronal reuptake blocker desipramine plus the extraneuronal uptake blocker deoxycorticosterone. They concluded that the effect seen on vessel contraction in the presence of perivascular fat was due to the dense sympathetic innervation in perivascular tissue.

Verlohren et al. were the first to publish studies on perivascular fat affecting the vascular responsiveness of mesenteric arteries (21). Their suggestion was that perivascular fat releases a vasodilator that mediates the observed reduction in sensitivity to a vasoconstrictor as well as a reduction in maximal contraction.

To eliminate the possibility of the reduced contration in the presence of perivascular fat was caused by the fat impeding diffusion of the constrictor, we applied fat around the vessel in a way that would leave a space above the vessel and thereby permitting direct access of the applied noradrenaline . We did observe a plateau for each dose of noradrenaline, which means that a steady state was reached. We found a reduced maximal contraction in the presence of surrounding fat; this cannot be explained by a diffusion hindrance, since this would only increase the time to steady state, not alter the equilibrium.

In their study, Verlohren et al. suggested that the vasodilator secreted by adipose tissue mediates its effect through opening of voltage-activated K+ channel in vascular smooth muscle cells. One way this was tested was by adding 4-aminopyridine (4-AP; a K+ channel blocker) to the bath in the presence of a vasoconstrictor; this counteracted the vasodilator action of perivascular fat. Again, the finding that another factor can modulate the effect of fat strongly argues against that the dilator effect of fat is due to a diffusion hindrance. The authors also measured the concentration of serotonin at different times, to rule out degradation of the vasoconstrictor in the presence of perivascular fat, and found the concentration to be constant over time. Uptake by adipose tissue or SNS nerve endings might cause extracellular agonist concentration around the smooth muscle cells to be lower in baths with (+)fat vessels. The experiment by Verlohren et al. would rule out the possibility of excessive uptake of vasoconstrictor that otherwise could be an explanation for a reduced response to a vasoconstrictor. Similarly our study showed that perivascular fat affects the maximal contraction, which cannot be explained by uptake of vasoconstrictor, since this is added in great excess. Thus, even though corticosterone is an inhibitor of extraneuronal uptake of noradrenaline, these experiments suggest that such an uptake is not important for the vasodilator action of perivascular fat, and that the effect of corticosterone on the vasodilation is not linked to extraneuronal uptake.

If the effect of perivascular fat on vascular contraction thus is not due to a hindrance for diffusion or an excessive uptake of the vasoconstrictor, a likely explanation is that a diffusible factor is secreted from adipose tissue. This factor could affect the smooth muscle cells directly or influence vasodilation by stimulating the endothelium to release NO. There are different reports as to whether the effect of fat is direct or NO-mediated (21)(6)(22). In preparations of aorta from rats, experiments have been performed where the bath solution from (+)fat vessels was transferred to (-)fat vessels and a vasodilation was observed(6). This clearly indicates that perivascular fat is actually releasing some kind of vasodilator substance. We have not yet been able to reproduce this experiment with transfer of solution from a (+)fat vessel to a bath with a (-)fat vessel, but so far we have attempted this only a few times. However, the fact that we initially have cleared all vessels from fat and then wrapped loose fat around the vessel also indicates a diffusible factor, since no other connection (such as nerves) between fat tissue and vessel can remain.

In the second part of this study we looked at how corticosterone would affect the vasodilation seen in the presence of perivascular fat. The result suggests that glucocorticoids partly antagonize the vasodilator effect of adipose tissue, although further experiments need to be done. While corticosterone seemed to increase sensitivity it did not reverse the reduction of maximal response seen upon addition of fat. However, such an effect could be masked by the fact that corticosterone by itself had a lowering effect on the maximal response in the absence of fat. In these first trials we used 10 µM corticosterone. A lower concentration might show different results, as could different incubation times. Glucocorticoid effects can be genomic (mediated by GR) and/or non-genomic(23). If the observed effect is due to a genomic effect the effect might develope more slowly. The time of incubation that we have used for these first experiments with corticosterone is inconclusive in this regard, so it is not possible to say if the effect is due to a regulation in transcription of a potent vasodilator or to an inhibition in the release of the same (24).

There are studies that describe an effect of cortisol on endothelial cells and smooth muscle cells in connection with vascular function. (25) (26). The studies generally conclude that corticosteroids have a tendency to make vessels more prone to contract either due to an effect on endothelial cells (decreasing the action of eNOS and iNOS as well as prostacyclin synthesis) or to a direct effect on vascular smooth muscle cells. Molnar et al. found that corticosterone has an immediate effect on the vascular response by enhancing vasoconstriction of rat aorta rings when phenylephrine was added to the bath solution(27). This is in contrast to the reduction in maximal response we observed when adding corticosterone to control vessels without fat.

Glucocorticoids as well as mineralocorticoids have been studied from many different angles concerning blood pressure and hypertension. It is well established that cortisol has affinity for both the GR (glucocorticoid receptor) and the MR (mineralocorticoid receptor) (28). Both receptors are present in adipose tissue(29)(30). The observed effect could thus be mediated through either receptor.

Several candidates for vasodilators have been suggested by different research groups. One of them is adiponectin. Several studies show that glucocorticoids affect the production and plasma concentration of adiponectin (7)(31)(32). Studies on a MR

antagonist's effect on preadipocytes show reduced expression of adiponectin as well as an increased expression of proinflammatory cytokines (33). Our preliminary result shows that there might be a connection between presence of cortisol in fat tissue and diminished effect of a product of adipose tissue that promotes vasodilation. Further studies need to be made in order to establish if there is a connection between high intracellular cortisol levels (14) in adipose tissue of obese, the secretion of a vasodilator substance, and the hypertension observed in obese (9).

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