Evidence for parasympathetic innervation of white adipose tissue, clearing up some vagaries

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TO THE EDITOR: Recently, we reported that fat tissue receives not only sympathetic but also parasympathetic innervation (5). Using microsurgery, transneuronal retrograde tracing, and hyperinsulinemic euglycemic clamps, we demonstrated that parasympathetic innervation of fat tissue has an anabolic effect and stimulated fat growth. In a follow-up paper on body fat distribution, we substantiated the evidence that the brain controls different body compartments by selective groups of neurons (6).

The Bartness group (4), interestingly, did observe parasympathetic innervation of adipose tissue using retrograde viral tracer labeling in the dorsal motor nucleus of vagus (DMV), but they question its relevance. In combination with the accompanying commentary of Berthoud et al. (2) the relevance, and even the presence, of the parasympathetic innervation were seriously doubted. As a basis for this conclusion, Giordano et al. (4) conducted two experiments in fat tissue, applying immunohistochemical and transneuronal retrograde tracing in combination with chemical sympathectomy. Fat tissue histology was negative for vesicular acetylcholine transporter (VACHT), vasoactive intestinal peptide (VIP), and neuronal nitric oxide synthase (nNOS). Moreover, Giordano et al. reported that fat pads did not get infected by PRV, if they were pretreated with the neurotoxin 6-hydroxy-dopamine (6OHDA). However, similar to what we reported in intact animals, evidence for parasympathetic innervation was observed by using PRV tracing.

From these results, Giordano et al. (4) and Berthoud et al. (2) concluded that parasympathetic innervation of fat tissue is not present to a significant extent. Although we understand that the absence of histological evidence for the presence of VACHT, VIP, and nNOS may lead to the conclusion that there is no vagal innervation, the authors should be well aware that the evidence for these markers has been very difficult to obtain in other organs and is highly variable. The liver, a structure with established vagal innervation, is an excellent example to illustrate this point (1, 3). In fact, by using the same marker for parasympathetic innervation as in the study of Giordano et al. (4) (i.e., VACHT) Schafer et al. (11) found very little staining in the liver, indicating that also for the liver, other parasympathetic markers will be more important. Interestingly, in the same paper of Schafer et al., VACHT was demonstrated in brown adipose tissue in the mediastinum, suggesting that also brown adipose tissue receives parasympathetic input. Among vagal transmitters that may play a role are pituitary adenylate cyclase-activating polypeptide (PACAP), leucine-enkephalin (L-ENK), and peptide histidine methionine (PHM) (8, 9, 14, 16). Since, in addition, paraffin-embedded sections of fat are known to lose much of their staining signal for these markers, the use of liver tissue as positive control for the same markers would have strengthened the point in the Giordano et al. (4) study that they are able to demonstrate parasympathetic markers. For their second point, the use of 6OHDA for chemical sympathectomy reduces significantly the transport of PRV by parasympathetic nerves (13). In addition, Giordano et al. (4) reported that the chemical sympathectomy of fat tissue is not complete: it only reduced the sympathetic neurotransmitter content by ~60%. In other words, 40% of the noradrenergic innervation is still present. No explanation is offered for this significant remaining input, but certainly these circumstances should be considered when interpreting the negative findings after such lesion experiments.

More notably, however, we have shown that the mechanical sympathectomy is essential to obtain significant vagal labeling by PRV. We explain this observation by the loss of sympathetic feedback, which probably results in the activation of parasympathetic neurons, as is evidenced by the higher uptake of PRV. This result is, in fact, confirmed by other studies of the Bartness group in which they showed that mechanical and chemical sympathetic denervation can induce fat growth (12). This result is in complete consonance with our findings, because the opposite happens after mechanical parasympathetic denervation; it results in lesser uptake of glucose in fat. The fact that we have observed that vagal neurons are more readily labeled after mechanical lesioning of the sympathetic nerve supports this reasoning. Berthoud et al. (2) also agrees with this point. However, in that editorial on the Giordano et al. (4) paper, the authors extensively discusses the possibility of leakage of PRV that might result in false-positive labeling. These concerns are understandable in view of the experiments with traditional retrograde tracers, where leakage into the peritoneum may induce some false-positive results. Moreover, Berthoud raises questions about bilateral labeling of the DMV and speculates that our intra-abdominal-injected tracers might have leaked to the organs in the peritoneal cavity. He also states that no intra-abdominal organs are reported to receive innervation from the ambiguous nucleus. The answer to these points is as follows: 1) we did inject totally denervated fat tissue with PRV and did not detect PRV in the central nervous system (CNS), let alone in the DMV (5); 2) we did put PRV on top of the fat pad within the intra-abdominal cavity and did not find any central tracer signal (5); 3) after sympathectomy of the left and vagotomy of the right retroperitoneal fat pad and injection of different PRVs in both, we did not find colocal-
ization of tracer in the CNS but separately labeled neurons (Kreier F, Veder L, Kalsbeek A, Sauerwein HP, Fliers E, Romijn JA, Mettenleiter TC, Buijs RM, unpublished observation); 4) double tracing from liver and intra-abdominal fat tissue revealed some neurons projecting to both liver and fat tissue and others projecting only to the liver, serving as an internal control against leakage (6); 5) no organ has been described that has unilateral vagal innervation; and 6) the colon and the pancreas have been reported to receive innervation by the ambiguus nucleus (10, 15).

In summary, we argue that on the basis of the experiments of Giordano et al. (4), it should not be concluded that parasympathetic innervation of fat tissue is absent. In fact, by this study and previous ones, the Bartness group has quite consistently reported figures of DMV labeling both from white and brown adipose tissue.

Finally, we have provided detailed photographs of the vagal and sympathetic innervation of retroperitoneal fat tissue in our last publication that will allow any skilled scientist to replicate our findings (6).

REFERENCES