Human obesity is associated with a chronic elevation in brain 5-hydroxytryptamine turnover

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ABSTRACT

The afferent signals that evoke changes in energy intake with regard to body weight regulation are presumed to arise partly from body stores, with the most likely candidate being adipose tissue depots. However, clinical investigation of the neuronal circuitry involved in the central nervous system’s processing of such satiety signals remains largely unexplored. Using percutaneously placed catheters in either the right or left internal jugular veins, we were able to quantify the release of central nervous system monoamine and indoleamine neurotransmitters in 64 weight-stable male subjects with varying degrees of adiposity. Veno-arterial plasma concentration differences and internal jugular blood or plasma flow were used, according to the Fick Principle, to quantify the amount of neurotransmitter stemming from the brain. By combining this technique with a noradrenaline and adrenaline isotope dilution method for examining neuronal transmitter release, we were able to examine the association between central nervous system neurotransmitters and efferent sympathetic nervous outflow and adrenomедullary function in human obesity. We found that brain 5-hydroxytryptamine (serotonin) turnover is chronically elevated in proportion to adiposity and is increased postprandially to a similar degree in lean and obese individuals. There was no difference in the degree of sympathetic nervous activity or rate of adrenaline secretion in the subjects examined. It therefore seems that in human obesity, in the face of a chronic elevation in peripheral satiety signals, brain serotonergic processes are switched on accordingly, but the subsequent physiological response involving a reduction in food intake, increased thermogenesis and sympathetic activity is in some way impeded.

INTRODUCTION

The afferent signals that evoke changes in energy intake with regard to body weight regulation are presumed to arise partly from body stores, with the most likely candidate being adipose tissue depots. Initially espoused in Kennedy’s lipostat or apidostat theory [1], this concept has gained increased credibility with the recent sequencing of the mouse obese gene and its human homologue [2]. Obesity results from an energy imbalance that could have its roots enconced in a diminution in energy expenditure, an increase in energy intake or a combination of both. Increased energy intake may have its basis in derangement of satiety regulation, which may arise as a result of a deficiency in production of a satiety
signal, defective coupling of satiety signals with the central nervous system, ineffective central nervous processing despite adequate satiety signals and effective coupling, or simply by an enhanced energy intake despite adequate and intact satiety regulatory systems. The observation that leptin levels are markedly increased in obese subjects [3,4] suggests that satiety signals are in fact intact in human obesity.

Disturbances of brain serotonergic, noradrenergic and peptidergic neuronal systems are implicated in the development of dietary problems such as stress-related eating, food craving and appetite disturbance [5]. The most powerful evidence supporting monoaminergic involvement in dietary control is that drugs used in the treatment of obesity, anorexia nervosa and bulimia tend to exert their effect by modulating the balance between serotonergic and noradrenergic neuronal activity [5]. Although only one of many neurotransmitters involved, the modulation of food intake by 5-hydroxytryptamine (serotonin) is not marginal; 5-hydroxytryptamine manipulations give rise to sizeable adjustments in feeding [6–9] such that enhanced central serotonergic activity is associated with a reduction in food intake.

Whereas a reduction in sympathetic nervous activity has been highlighted as a potential mechanism predisposing to body weight gain [10], activation of the sympathetic nervous system in obese individuals may help to stabilize body weight and restore energy balance by driving thermogenesis [11]. Interestingly, previous reports, conducted in human subjects, have illustrated some dependence of sympathetic outflow on cerebral noradrenergic activity [12,13]. With this in mind we sought to examine the brain’s processing of the afferent information involved in the maintenance of body weight and energy intake in obese and lean individuals, and to investigate the possible involvement of aberrations in brain serotonergic, noradrenergic and the sympathetic nervous systems in the generation of obesity.

METHODS

Using a novel technique, based on earlier studies performed in non-human primates [14], and refined and validated for use in a clinical setting [12,15], we examined brain monoaminergic neuronal activity in 64 weight-stable male volunteers (mean age 35 ± 2 years). The protocol presented in this report conformed to the relevant guidelines of the National Health and Medical Research Council of Australia and was approved by the Alfred Hospital Human Research Ethics Committee. All volunteers gave written informed consent before their participation in the experimental procedure. Data were obtained from subjects encompassing a broad spectrum of body types: from those with a body mass index (BMI) within the healthy range to those with values indicative of moderate obesity (range 20.1–35.6).

Subjects

The subjects were recruited either by local advertisement or, in the case of the majority of the overweight subjects, utilizing the database of a weight reduction centre (Gutbusters, Melbourne, Australia). All subjects underwent a comprehensive clinical and physical examination to screen for any previously undiagnosed medical conditions before their acceptance in the experimental protocol. Exclusion criteria for all studies included a history of major illness, cardiovascular disease, current drug medication and previous psychiatric therapy. The screening procedure entailed a full blood examination including leucocyte differential analysis, serum biochemistry and tests for previous exposure to the hepatitis B and human immunodeficiency viruses. All overweight subjects recruited from the weight reduction centre had undergone the programme for at least 1 year before experimentation but had regained their original body weights. Each subject’s height and weight was recorded and their BMI computed. Fat mass was predicted from individual BMI values using the age- and gender-specific equations of Womersley and Durnin [16].

General procedure

All studies were performed with subjects in the supine position after an overnight fast. Caffeinated beverages, alcohol and tobacco smoking were prohibited for the 12 h preceding the catheter study. At 08:00 hours on the morning of the experimental protocol, subjects had a standardized light breakfast, typically comprising fruit juice and toast. Blood samples were obtained from central venous and arterial catheters percutaneously inserted under strict aseptic conditions in the cardiac catheterization laboratory of the Alfred and Baker Medical Unit according to methods described previously [17].

After skin preparation and adequate local anaesthesia, an arterial catheter was inserted percutaneously into a brachial artery. The arterial catheter was connected to a continuous infusion of 5% dextrose containing 4 units/ml heparin via an intraflow adapter and pressure bag, which ensured delivery of 3 ml/h at a bag pressure of 300 mmHg, if the system was not flushed manually. Intra-arterial pressure was recorded continuously via a Spacelabs Inc. model 90603 2-channel pressure monitor (Redmond, WA, U.S.A.). With fluoroscopic control, a 7F type CCS-7U coronary sinus thermodilution catheter (Webster Laboratories, CA, U.S.A.) was positioned in the internal jugular vein, beyond the mandibular angle, upstream to points of entry of veins draining the face and neck to minimize any contamination of the cerebral...
venous effluent. The catheter position was verified with 2 ml of radio-opaque contrast medium (Omnipaque, Winthrop Pharmaceuticals, NY, U.S.A.). The catheter was used for sampling internal jugular vein blood and for the determination of internal jugular blood flow by thermodilution [17,18]. Throughout the course of the catheter studies, tracer doses of L-[7–3H]noradrenaline (specific activity 11–25 Ci/mmol, New England Nuclear, Boston, MA, U.S.A.) and 3H-labelled adrenaline (L-[N-methyl-3H]adrenaline, specific activity 69–78 Ci/mmol, New England Nuclear) were infused via a peripheral hand vein for the assessment of plasma noradrenaline and adrenaline kinetics [19,20].

In a subset of the individuals, six lean [mean BMI 23.6 (range 22.2–25.5)] and six obese [mean BMI 31.0 (range 28.1–35.6)], we also examined 5-hydroxyindoleacetic acid (5-HIAA) plasma kinetics [21]. After the procurement of resting internal jugular venous blood samples, further samples were obtained from the kidney using the same central venous catheter repositioned to sample from the right renal vein. Renal plasma flow was estimated by measuring the rate of plasma clearance of para-aminohippuric acid (PAH) [22]. Effective renal plasma flow was derived by dividing the clearance rate for PAH by its renal extraction (obtained by determining the arterial and renal venous PAH concentrations simultaneously).

Given that central nervous system serotonergic mechanisms have been evoked in the mediation of satiety [6] and that obese gene expression is increased in rats after feeding [23], we thought it pertinent to investigate the brain’s responses after the ingestion of a moderately sized mixed meal. Matching internal jugular venous and arterial blood samples were obtained in 11 subjects, 5 lean [mean BMI 23.6 (range 22.2–25.5)] and 6 obese [mean BMI 31.0 (range 28.1–35.6)], before and after (75 ± 3 min) meal ingestion of a commercially available liquid meal of 41.84 kJ/kg lean body mass as determined from skinfold (biceps, triceps, subcapular and suprailiac) thickness.

In all studies, blood samples for plasma neurochemical evaluation were obtained simultaneously from the arterial and venous catheters and immediately placed in tubes containing an anticoagulant/antioxidant mixture. At the completion of the catheter study and within 15–75 min of sampling, the blood samples were centrifuged at approximately 1000 g for 30 min at 4 °C to avoid disruption of blood platelets [24] and plasma was stored at –80 °C until assayed.

### Assessment of central nervous system monoaminergic activity

Veno-arterial plasma concentration differences combined with an appropriate internal jugular vein flow measurement were used, according to the Fick Principle, to determine metabolite overflows from the brain which were used as indicators of neuronal activity and were calculated according to the following general formula:

\[
\text{Overflow} = (\text{Venous}_{\text{concn.}} - \text{Arterial}_{\text{concn.}}) \times Q
\]

where Venous$_{\text{concn.}}$ and Arterial$_{\text{concn.}}$ are the plasma concentrations of the compound of interest in the venous effluent and the arterial blood supply respectively, and Q refers to the plasma or blood flow, adjusted where appropriate according to the distribution of the compound between blood cells and plasma, which differs for measured neurotransmitters and metabolites [17,25]. For the catecholamine, noradrenaline, a further adjustment was made allowing for the fractional extraction of 3H-labelled catecholamine across the brain during a constant rate infusion of radiolabelled noradrenaline [26]. Internal jugular venous–arterial plasma concentration gradients of the principal 5-hydroxytryptamine metabolite, 5-HIAA, were used as an estimate of brain 5-hydroxytryptamine turnover while veno-arterial concentration differences of noradrenaline and its lipophilic metabolites, dihydroxyphenylglycol and 3-methoxy-4-

<table>
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<tr>
<th>Table</th>
<th>Body composition, haemodynamic and sympathoadrenal data in subjects with varying degrees of adiposity</th>
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<tr>
<td>NA, noradrenaline; ADR, adrenaline. n = 16 in each group.</td>
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<td>Fat mass percentile…</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Fat mass (kg)</td>
<td>10.5 ± 0.3</td>
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<td>Mean arterial pressure (mmHg)</td>
<td>90 ± 2</td>
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<td>Heart rate (beats/min)</td>
<td>64 ± 2</td>
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<tr>
<td>Total NA spillover (nmol/min)</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>Total ADR secretion (nmol/min)</td>
<td>1.3 ± 0.2</td>
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hydroxyphenylglycol, were used as an index of brain noradrenaline turnover.

**Neurochemical assays**
Plasma neurochemical concentrations were determined by HPLC coupled with electrochemical detection according to established techniques [15].

**Statistical analysis**
All results, unless otherwise specified, are expressed as means ± S.E.M. Comparisons between groups were evaluated using one-way analysis of variance. Relationships between variables were evaluated with least-squares linear regression analysis. The null hypothesis was rejected at $P < 0.05$.

**RESULTS**
The degree of adiposity of the individuals was significantly related, albeit weakly, to their age (BMI = 0.04 age + 23.4; $r = 0.20, P < 0.05$) but not to their degree of sympathetic nervous activation, blood pressure, heart rate or magnitude of adrenaline secretion (Table 1). Positive internal jugular venous–arterial plasma concentration gradients of noradrenaline and its lipophilic metabolites and of 5-HIAA were found across the brain. Although the internal jugular venous overflow of these compounds bore no relation to the age of the subjects, examination of the data in terms of degree of adiposity revealed a marked elevation in 5-HIAA overflow from the brain in those subjects with the highest fat mass (Figure 1). The estimated central nervous system turnover of noradrenaline was identical in subjects of varying body types (Figure 1). Given that the internal jugular vein blood flows were identical in all groups of subjects the differences in brain 5-HIAA production in the obese individuals were attributable to an elevation in the internal jugular vein–arterial plasma 5-HIAA concentration gradient ($y = 0.3 \times -3.3; r = 0.51, P < 0.001$).

To assess whether the measured increase in brain 5-HIAA overflow represented a specific central nervous system activation of 5-hydroxytryptamine-containing cell groups or was part of a more widespread increase in whole-body serotonergic activity, we examined 5-HIAA plasma kinetics in a subset of lean and obese subjects. In the obese individuals the minimum steady-state rate of whole-body 5-HIAA production, estimated from the renal 5-HIAA extraction, was in fact substantially reduced compared with that of the lean subjects (13 ± 1 versus 21 ± 2 nmol/min, $P < 0.05$). Thus, the enhanced release of 5-HIAA into the cerebral venous effluent in the obese subjects (1.5 ± 0.7 nmol/min) represented a proportionally greater contribution made by the brain to the total 5-HIAA plasma pool (12% in the obese subjects versus 1% in the lean individuals).

After meal ingestion there was an incremental rise in the internal jugular venous–arterial 5-HIAA plasma concentration gradient (Figure 2). This translated into a greater than doubling of the 5-HIAA stemming from the brain (0.4 ± 0.2 versus 1.1 ± 0.5 nmol/min). There was no difference between the responses in lean or obese

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**Figure 1** Estimated central nervous system turnover of 5-hydroxytryptamine (top) and noradrenaline (middle) and the magnitude of internal jugular venous blood flow (bottom) in subjects with varying degrees of fat mass ($n = 16$ in each group)
Values are expressed as means ± S.E.M.
**DISCUSSION**

In line with the observation that leptin levels are markedly increased in obese subjects [3,4], our principal finding, that brain 5-hydroxytryptamine turnover is related to fat mass, suggests that satiety signalling related to body fat stores is not deficient in obese individuals. Given the similar degrees of sympathetic activity in lean and obese subjects, the increased 5-hydroxytryptamine turnover that we measured may represent the central nervous system’s response to a chronic, but perhaps futile, satiety signal. Although our observations indicate that whole-body sympathetic nervous function is normal in obese subjects, we cannot discount the presence of potentially relevant alterations in regional sympathetic nervous activity [27].

The activity of central nervous system monoaminergic and peptidergic neuronal systems is implicit in the regulation of dietary control and thermogenesis. Indeed, dysfunction of brain 5-hydroxytryptamine, noradrenaline and neuropeptide Y systems is implicated in the development of obesity and appetite disturbances [5]. Mice deficient in the 5-HT1c receptor are overweight as a result of abnormal control of feeding [28] and the hypophagic effect of sibutramine is related to its ability to inhibit the re-uptake of both noradrenaline and 5-hydroxytryptamine [29]. Interestingly, there is evidence that neuropeptide Y-induced feeding is antagonized after stimulation of hypothalamic serotonic receptors [30,31]. Although we have been unable to document the release of neuropeptide Y from the brain in human subjects [32], previous reports have demonstrated the release of noradrenaline and its metabolites into the cerebrovascular venous effluent and have illustrated the dependence of sympathetic outflow on cerebral noradrenergic activity [12]. A reduction in sympathetic nervous activity has been implicated in the genesis of animal models of obesity [33] and has been highlighted as a potential mechanism predisposing to body weight gain in humans [10]. Such views are in accordance with the apparent importance of the sympathetic nervous system in virtually all the individual components of daily energy expenditure. Although we cannot discount the coexistence of some form of down-regulation of central nervous system serotonic receptor-mediated mechanisms, the normality of brain noradrenaline turnover and sympathetic nervous activity in the obese, in the face of elevated brain serotonic activity, suggests that communication between central nervous serotonic and noradrenergic neurons is in some way impaired in these individuals.

Our results suggest that long-term, adiposity-related, satiety signals share a common neurotransmitter with shorter-term, feeding-associated, signals. On the one hand, feeding-associated signals are generated according to the sensory qualities of the ingested meal, with the integration of metabolic information involving multiple brain regions utilizing a variety of neurotransmitters. For example, noradrenergic, γ-aminobutyric acid- and neuropeptide Y-containing neurons in the paraventricular nucleus of the hypothalamus form a neuronal network whose function is to selectively potentiate the ingestion of carbohydrate, an action antagonized by 5-hydroxytryptamine (for review see [5]). On the other hand, the afferent signals that evoke changes in energy intake with regard to body weight regulation are presumed to arise from adipose tissue depots. Again, the hypothalamus is thought to be pivotal in the integration of these signals and the normal physiological response to such stimulation is believed to involve activation of the sympathetic nervous system with concomitant increases in thermogenesis and energy dissipation. While our results point to serotonic involvement, neuropeptide Y-containing neurons are also implicated in the processing of such information [34]. Whether the integration of such long- and short-term signals utilizes a common pathway encompassing identical neuronal circuitry is problematic at this stage, but the potential for hormones such as insulin, cholecystokinin or corticoesterone to act as mediators between the periphery and the brain deserves further attention.

Observations that the levels of polyunsaturated fatty acids in plasma are predictive of cerebrospinal fluid 5-
HIAA [35] and that brain levels of tryptophan and 5-hydroxytryptamine vary in the acute postprandial period according to the macronutrient composition of the ingested meal [36] raises the possibility that increased brain 5-hydroxytryptamine turnover in obese subjects results as a consequence of diet selection rather than elevated adiposity per se. Given the inherent difficulties in obtaining habitual dietary intakes, coupled with the generally accepted view that obese subjects frequently under-report on self-recorded diet inventories [37], it is difficult to address this issue with certainty. The fact that our examinations were performed after an overnight fast precludes the possible interference of recently ingested macronutrients in our measurements. We were unable to obtain habitual dietary intakes on all subjects and did not routinely measure plasma fatty acid levels; hence we cannot exclude with certainty the possible confounding influence of habitual diet on our measurements.

Our results are seemingly at odds with the knowledge that drugs such as d-fenfluramine are effective in the management of obesity. However, criticism on such grounds presupposes that drugs of this ilk exert their effect via a purely serotonergic mechanism and ignores results documenting interactions between a variety of central nervous system neuronal pathways after fenfluramine administration [38,39]. Moreover, chronic fenfluramine treatment results not only in the down-regulation of the 5-hydroxytryptamine uptake carrier, but in a progressive reduction in fenfluramine-stimulated 5-[3H]-hydroxytryptamine release from brain synaptosome preparations [40].

On the face of it, the recent observations by Strombom et al. [41], indicating that obese women exhibit reduced levels of 5-HIAA in cerebrospinal fluid, seem also to contradict the findings of the present report. Given the marked 5-HIAA concentration gradient between cisternal and lumbar cerebrospinal fluid [42] and the prominent serotonergic innervation of the spinal cord [43], whether lumbar cerebrospinal fluid concentrations are a reliable indicator of brain events is open to some conjecture. The demonstration that extracellular 5-hydroxytryptamine levels in the forebrain are subject to a degree of gender and hormonal specificity is also pertinent in the present context [44].

While acknowledging that other neuronal systems are also involved in satiety, our observations that brain 5-hydroxytryptamine turnover is increased postprandially and is chronically elevated in proportion to the degree of adiposity, indicate that 5-hydroxytryptamine-mediated satiety-related central nervous system processing is not impaired in the obese, and that satiety signalling related to body stores is also not deficient in obese individuals. This study for the first time provides a physiological tool for the examination of brain processing of satiety in humans, and the application of this technique may help to answer current issues, including those related to leptin, concerning the central nervous system’s role in the regulation of satiety and the maintenance of body mass.

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