Aspirin as a promoter of ephedrine-induced thermogenesis: potential use in the treatment of obesity\textsuperscript{1,2}

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ABSTRACT Chronic administration of aspirin to obese mice had no effect on energy balance and body composition. In contrast, ephedrine increased energy expenditure by 9% and reduced body weight and body fat by 18% and 50%, respectively: obesity, however, was reduced but not reversed. In the presence of both ephedrine and aspirin, increase in energy expenditure found during treatment with ephedrine alone was doubled, and the obese group lost > 75% of body fat: obesity was reversed. These studies indicate that although aspirin administered alone has no influence on energy balance it can markedly potentiate thermogenic properties of ephedrine, effects which led to a normalization of body composition of the obese to that of the lean. Such ephedrine-aspirin mixtures, often found in over-the-counter preparations for asthma and bronchial disorders, could be put to new use as aids for treatment of human obesity. Am J Clin Nutr 1987;45:564–9.

KEY WORDS Obesity, thermogenesis, aspirin, ephedrine, energy balance, VMH obesity, CFLP mice

Introduction

Current research in obesity treatment is experiencing a resurgence of interest in thermogenic drugs, ie, drugs that increase metabolic rate and induce loss of body fat. The emphasis, however, is no longer directed at the development of safer and more effective thyroid derivatives or uncouplers of oxidative phosphorylation, but has shifted towards an active search for thermogenic stimulants of the sympathetic nervous system (SNS). This new approach follows several lines of evidence, mostly from animal studies, that indicate an impairment in activity of SNS as contributory to the etiology of obesity. An insufficiency of sympathetically released noradrenaline (NA) (1–6), rather than a defective response to thermogenic effects of the neurotransmitter (7, 8), is thought to be responsible for the elevated energy efficiency in the obese. Drugs that mimic activity of SNS and increase metabolic rate to induce reduction in body fat, therefore, offer considerable therapeutic potential and provide a rational approach for treatment.

It did not take long before a number of novel \(\beta\)-agonists (9), some with specificity for brown adipose tissue (10), as well as a wide variety of sympathomimetics currently used for other treatments (11) were reported to possess thermogenic anti-obesity properties. In particular, the ability of ephedrine to increase metabolic rate and to reduce body fat is well-established in several animal models of obesity and in different laboratories (11–13). We now report on the thermogenic effects of ephedrine in combination with aspirin.

Materials and Methods

Mice of the CFLP strain were made obese by chemical lesioning of the hypothalamus following injections of monosodium glutamate (MSG) during the first week of life. Both MSG-injected mice and lean controls were weaned and maintained on a stock diet (CRM, Christopher Hill Group, London): obesity develops without hyperphagia in the ad libitum-fed MSG-treated animals and results entirely from an elevated efficiency of energy utilization (14). Energy balance studies over a period of 6 wk

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were then conducted on age 22–23 wk male obese mice, which were put one to a cage and housed in a metabolism room at 25 ± 1°C with a 12:12 light-dark cycle. At the start of the experiment, 30 obese mice were divided into five groups (n = 6) with similar mean body weights. One group was killed at the beginning to provide initial carcass energy content. Another group, the no-drug control group, was fed a powdered form of the stock diet, and the remaining three groups were fed the same diet with either ephedrine hydrochloride (Thorntons and Ross, Linthwaite Laboratories, UK) or aspirin (Boots Co, Nottingham, UK) or both in combination. Ephedrine and aspirin were administered at doses of 1.0 and 8.0 g/kg diet, respectively, ie, in approximately the same dose ratio as found in several preparations for asthma, cough, and bronchospasm.

The method of administering the drugs, incorporation in a diet fed ad lib, has several advantages over more traditional routes employed by pharmacologists. Firstly, considering the long-term nature of such nutritional investigations, it avoids frequent daily handling of animals for injection or oral gavage and reduces the stress imposed on the animals. Secondly, this method ensures that the drug is administered at the same time as food is eaten and reduces the likelihood of drug-induced potention of thermogenesis associated with food. Thirdly, it overcomes the problem of deciding whether to dose the animals per kg body weight, per kg to the power of 0.75, or per lean body mass: such considerations are clearly important when dealing with obese animals of different body sizes and different degrees of obesity. Finally, this method of stating dose rates is more relevant when transferring the results of animal metabolic experiments to man, since interspecies metabolic rate is more closely related to energy intake than to body weight (15–17). It is possible to calculate the doses given in this paper in terms of mg/kg body weight from the data provided, but it is our experience that animals given drugs in their food can tolerate higher doses than when the drug is administered acutely by injection or oral gavage and, hence, comparison with other work may be misleading.

Food intake was measured on a weekly basis throughout the experiment, and the metabolizable energy (ME) intake was calculated by the method of Miller and Payne (18). At the end of the study, all animals were killed by cervical dislocation. The skull and the thoracic and abdominal cavities were incised and carcasses were dried before homogenization. Triplicate samples of each carcass were analyzed for energy content by bomb calorimetry (18). Carcass fat was measured by the soxhlet fat extraction method (19) on duplicate samples of dried and homogenized carcasses. Carcass protein was calculated from a general formula relating energy derived from fat, total energy value of the carcass, and energy derived from protein (5). Body energy gain was calculated from the difference between final body energy content and energy content of the initial control group killed at the beginning of the experiment. Energy expenditure (total heat production) over the entire 6-wk study was the difference between ME intake and energy gain (ie, by the comparative carcass technique). Oxygen consumption over 24-h periods was measured at 25 ± 1°C with twin indirect calorimeters described previously (6) during weeks 3–6 of the experiment.

Data were analyzed by analysis of variance (ANOVA) and by the Newman-Keul's multiple sample comparison test with a probability level of < 0.01 (20).

The United Kingdom Home Office Regulations and Guide for the Care and Use of Laboratory Animals were followed.

Results

Growth curves of the groups of obese animals are shown in Figure 1. The no-drug control group gained weight steadily and administration of aspirin had no effect on rate of weight gain nor on food consumption when compared with the no-drug control group. On the other hand, effects of ephedrine on body weight and food intake are consistent with other reported studies in this animal model: an initial anorectic effect contributes, at least partially, to rapid loss in weight over the first 2 wk, but over subsequent weeks this lower body weight is maintained despite the fact that food intake is similar to or slightly higher than that of the control group. Administration of ephedrine with aspirin shows similar effect to that of ephedrine alone on the level and pattern of food intake, but losses in body weight are more pronounced with the ephedrine-aspirin mixture throughout the study.

Over the 6-wk experimental period, the ephedrine- and ephedrine-aspirin-treated groups lost 18% and 27% of body weight, respectively, relative to the control group (p < 0.001), although there are no significant differences in total food intake among groups (Table 1). Energy balance data indicate that aspirin alone has no effect on metabolic rate, whereas both ephedrine and ephedrine-aspirin mixture increase total heat production significantly by 9% and 17%, respectively, and induce marked losses in body energy stores. Potentiation of the thermogenic effects of ephedrine by aspirin is confirmed by data on 24-h oxygen consumption that also show that increases in metabolic rate with the mixture are nearly twice as much as with ephedrine alone (whether data are expressed per animal or per metabolic body size).

Analysis of body composition (Fig 2) indicated that ephedrine or the ephedrine-aspirin mixture had no effect on body protein, but that the thermogenic effects of the combination resulted almost entirely in reducing body fat: compared with the no-drug controls, total body fat was reduced by 50% in the ephedrine-treated group and by > 75% in the ephedrine-aspirin-treated group. In contrast, treatment
FIG 1. Body weight and food intake of obese mice during treatment for 6 wk with aspirin (■), ephedrine (□), or both aspirin and ephedrine (●), compared with a no-drug control group (○). The vertical bars represent the standard errors of mean values (n = 6). ME intake for 2-wk periods is represented by bar charts where each bar represents the mean of two measurements of 1 wk each. ANOVA indicates significant differences (p < 0.001) in body weight from wk 1 onwards with multiple sample comparison test showing significant differences (p < 0.01) between ○, ■ vs □, ● and also between □ and ●. ANOVA indicates significant differences (p < 0.01) in food intake only during the first 2 wk with multiple comparison test showing significant differences (p < 0.01) only between ○, ● vs □, ■ during that period. In contrast, ANOVA shows no significant differences in food intake during wk 3–4 and 5–6 or for total (cumulative) food intake over the entire 6-wk study.

with aspirin alone had no effect on body composition. As shown in Figure 2, there are no significant differences in total body energy, fat, or protein content between the no-drug control group and the aspirin-treated group and between the lean control group and the ephedrine-aspirin obese group: the MSG-induced obesity is completely reversed by the sympathomimetic mixture.

Discussion

There are now several sympathomimetic drugs, both new and old, that have been shown to possess thermogenic effects, which contribute to fat reduction in obese animal models (9–13). However, none of them have yet been shown to be capable of completely reversing obesity. This is well illustrated for ephedrine, which as one of the most potent thermogenic sympathomimetic stimulants yet reported reduces body fat of the obese by half: the treated animals, however, are still obese since they have at least 3 times more body fat than the lean animals have (Fig 2). The ability of aspirin to potentiate the thermogenic effects of ephedrine is remarkable because the body energy stores and body fat content of the obese are completely normalized to that of lean levels (Fig 2). Such a reversal of obesity occurs without loss of body protein indicating that thermogenic effects of the ephedrine-aspirin mixture are entirely lipid specific.
SYMPATHETIC STIMULATION OF THERMOGENESIS

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Six-week energy balance and 24-h oxygen consumption of obese mice treated with aspirin, ephedrine, or both aspirin and ephedrine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No-drug (a)</td>
</tr>
<tr>
<td>ME intake (kJ/mouse)</td>
<td>2685 ±42</td>
</tr>
<tr>
<td>Energy gain (kJ/mouse)</td>
<td>177 ±26</td>
</tr>
<tr>
<td>Energy expenditure (kJ/mouse)</td>
<td>2508 ±42</td>
</tr>
<tr>
<td>24-h oxygen consumption (L O2/mouse)</td>
<td>2.74 ±0.05</td>
</tr>
<tr>
<td>(L O2/kg0.75)</td>
<td>31.4 ±0.3</td>
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</table>

* All values are mean ± SE.

The exact mechanism by which ephedrine chronically stimulates thermogenesis, let alone its potentiation by aspirin, is uncertain although direct postsynaptic adrenoceptor stimulation (21) and enhancement in NA release from the sympathetic nerve terminals are.

![Fat and Protein](image)

**FIG 2.** Body energy stores compartmented into fat (blank area) and protein (dark area) of the no-drug obese control group (a), the obese groups treated with aspirin (b) or ephedrine (c) or both (d) and the lean control group (e). The vertical bars represent the standard errors of mean values (n = 6). ANOVA indicates significant differences (p < 0.001) between groups for body fat and body energy content, but not for body protein. Multiple comparison tests show significant differences (p < 0.001) in body fat and energy content between a, b vs c, d, e and between c vs d, e; there are no significant differences in body composition between a, b and between c and d.
probably involved. Activity of SNS is believed to be modulated by a number of feedback mechanisms including NA itself, adenosine, and prostaglandins, which, when NA levels in the synaptic cleft increase, would exert an inhibitory effect on presynaptic sites to reduce further NA secretion (22). Thus, the effect of ephedrine in enhancing the release of NA from the sympathetic terminals may be sustained by the ability of aspirin to inhibit prostaglandin biosynthesis. Although purely speculative, this explanation could provide a mechanism by which aspirin can potentiate thermogenic effects of ephedrine. A similar mechanism based on the ability of methylxanthines to inhibit adenosine has recently been proposed to explain potentiative effects of caffeine or theophylline on ephedrine-induced thermogenesis, effects which also lead to a reversal of obesity in this same animal model (23). Thus, the effects of ephedrine in combination with aspirin or methylxanthines could be said to be that of a long-acting NA which corrects defective thermogenesis in animals that become obese following lesions in hypothalamus.

The major site of ephedrine-induced thermogenesis is debatable, but both brown adipose tissue (24) and skeletal muscle (25) have been implicated. Synergism between caffeine and an isomer of ephedrine (phenylpropanolamine) in increasing brown fat thermogenesis has been demonstrated in acute in vivo studies on lean rats (26). Although a central stimulatory effect of these drugs on thermogenesis cannot be disregarded, it is likely that many of the anti-obesity effects of ephedrine in combination with aspirin (or methylxanthines) are from an increased and sustained thermogenic effect of peripherally released NA on brown adipose tissue and skeletal muscle.

In man several studies have shown that single-dose administration of ephedrine increases metabolic rate (27–30) and that during chronic administration thermogenic effect of ephedrine is enhanced (29) and is associated with increased lipid oxidation (30); these effects could, at least partially, account for losses in body weight when ephedrine is administered over a few weeks (29, 31). Given the marked potentiative effect of aspirin (or methylxanthines) on the thermogenic effects of ephedrine, these mixtures may have a major potential as aids in treatment of human obesity. They may be presumed to be relatively safe if only because they have been part of traditional medicine for centuries: in many countries they are currently available over the counter.

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References

SYMPATHETIC STIMULATION OF THERMOGENESIS