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Salbutamol Antagonizes Insulin- and Sodium Mercaptoacetate-Induced But Not 2-Deoxy-D-Glucose-Induced Hyperphagia

ENZO NISOLI,* VITTORIO GAROSI,* JOHN E. BLUNDELL† AND MICHELE O. CARRUBA*[1]

*Department of Pharmacology, Chemotherapy and Medical Toxicology, School of Medicine, University of Milan, 20129 Milan, Italy and †BioPsychology Group, Psychology Department, University of Leeds, LS2 9JT, England

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NISOLI, E., V. GAROSI, J. E. BLUNDELL AND M. O. CARRUBA. *Salbutamol antagonizes insulin- and sodium mercaptoacetate-induced but not 2-deoxy-D-glucose-induced hyperphagia.* PHARMACOL BIOCHEM BEHAV **54**(2) 409–413, 1996. — The role of beta-adrenoreceptors in modulating feeding in glucoprivation- and lipoprivation-induced hyperphagias was studied in rats by measuring the efficacy of the selective $beta_2$-adrenoreceptor agonist salbutamol to antagonize the hyperphagic response induced by injection of 2-deoxy-D-glucose (2-DG), insulin, or sodium mercaptoacetate (MA). 2-DG and insulin are blockers of glucose utilization, and their administration stimulates receptor cells that are selectively sensitive to central glucose availability. MA stimulates feeding in rats maintained on a fat-supplemented diet, by blocking fatty acid oxidation at different levels in the metabolic pathway. We found that salbutamol dose-dependently antagonized both the insulin- and MA-induced hyperphagia, with reductions in food intake up to 100% compared with rats treated with insulin or MA alone. On the contrary, salbutamol, even at the highest dose (15 mg/kg, IP), was completely ineffective against 2-DG-induced hyperphagia. The present results support the previously proposed notion that there are different neuronal or humoral circuits underlying the hyperphagic responses to the metabolic stimuli induced by glucoprivation (i.e., 2-DG and insulin administration), and they extend our knowledge on the effects of salbutamol on glucoprivic and lipoprivic control of feeding.

Experimentally induced hyperphagia Anorectic drugs 2-Deoxy-D-glucose Insulin Sodium mercaptoacetate Salbutamol Neural pathways

DURING recent years brain monoamines have been shown to play key roles in feeding behavior (3,10,17). It has been shown that activation of beta-adrenergic sites in the perifornical area of the lateral hypothalamus (PFH), by direct or indirect beta-adrenergic agonists, causes anorexia in rats. Indeed, intra-PFH injection of norepinephrine, isoproterenol, and amphetamine reduced food intake (16). This inhibitory effect was prevented by beta-adrenergic blockers, such as propranolol, but not by alpha-adrenergic blockers, such as phentolamine, indicating that beta-adrenoceptors are involved in the anorectic effect of the adrenergic agonists (18). In addition, it has been reported that systemically or centrally administered beta-adrenergic agonists can effectively reduce food intake by stimulating the central beta-adrenergic sites (14,27). Salbutamol, a selective $beta_2$-adrenergic agonist, induced anorexia when injected peripherally or into the PFH (15). This reduction was prevented by the systemic administration of propranolol (4).

Central or systemic administration of glucose antimetabolites, such as 2-deoxy-D-glucose (2-DG) or 5-thio-D-glucose, or hypoglycaemic doses of insulin stimulate feeding by activating receptor cells that are selectively sensitive to central glucose availability (20,21,28). A recently described second metabolic control of feeding behavior induces an increase in rat food consumption, as a consequence of the blockade of fatty acid oxidation (i.e., lipoprivation). Compounds, such as sodium mercaptoacetate (MA) and methylpalmoxirate, which block

[1] Requests for reprints should be addressed to Prof. Michele O. Carruba, Dipartimento di Farmacologia, Chemioterapia e Tossicologia Medica, Università degli Studi, Via Vanvitelli, 32, 20129-Milano, Italy.

fatty acid oxidation at different levels in the metabolic pathway (1), stimulate feeding (25). This effect was most clearly evident in rats adapted to eat a fat-supplemented diet (25).

Thus, changes in the peripheral glucose or lipid metabolism seem to activate homeostatic mechanisms leading to behavioral operations. The neuronal networks underlying these homeostatic behavioral adaptations are beginning to be mapped out (6,24).

Because it is well known that feeding behavior can be modulated in an inhibitory fashion by different drugs acting on specific neurotransmitter systems (8,10), the study of the ability of these drugs to influence the feeding response to metabolic changes can draw some light on the neuronal organization of the brain systems involved in these behavioral adaptations.

Our previous work showed that different neuronal or humoral circuits underlie 2-DG- and insulin-induced eating (7). Indeed, whereas 2-DG needs functionally operating alpha$_2$-adrenoceptors to bring about increased food intake, insulin does not (7,8). In addition, serotoninergic and dopaminergic anorectic drugs modulate the eating responses of rats to cerebral glucoprivation induced by 2-DG or by insulin differently (7). These differential activities provide clues about the neurochemical coding subserving each type of hyperphagia.

Because our more recent work has reported that beta$_2$-adrenergic agonists, such as salbutamol, antagonized lipoprivic feeding (12), in the present study we have compared the effect of beta$_2$-adrenoceptor stimulation on 2-DG- and insulin-induced hyperphagias to MA-induced feeding. This experimental approach allows analyzing the effects of salbutamol on glucoprivation-induced feeding, giving some insight on the central neural pathways involved.

METHOD

Animals and Housing Conditions

Experiments were performed on adult male Sprague–Dawley rats of 275–300 g body weight obtained from Charles River (Calco, Como, Italy). They were housed in groups of three in makrolon cages (22 × 17 × 13 cm) upon arrival in a temperature-controlled room (21 ± 0.5°C) on a 12 L : 12 D cycle (lights on 0700 h). All behavioral testing was done in the rats' home cages. Food (see below for special diet) and tap water were available ad lib throughout the 24 h.

Rats were fed either a standard (pelletted lab chow, 3.33% fat) or a fat diet (18% fat; Laboratori Piccioni, Italy; for composition see Table 1). These foods were provided to the animals in nonspill feeding cups. The rats were adapted to diet and maintainance conditions for 2 weeks before experimentation. The fat diet used in our studies was formulated according to the recipe of Scharrer and Langhans (25) and was shown in their studies to be adequate for expression of a feeding response to MA-induced blockade of fat metabolism. The diets supplied were isocaloric (~ 16.5 kJ/g). Feeding tests were conducted beginning 2 h after the beginning of the light period. Groups of rats were matched for body weight and food intake, which were always measured during the preceding light period.

Feeding Tests

In Experiment 1, rats housed in individual cages the day before were divided into groups of six animals each. The control group received an IP injection of saline, whereas the experimental groups were injected IP with the same volume of 2-DG (250, 500, 750 mg/kg) or insulin (3, 6, 9 IU/kg) solutions, used to block glucose utilization in rats fed on standard diet, or MA (23, 46, 69 mg/kg), used to block fatty acid oxidation in rats fed on standard or fat diet. Cumulative intake of the diet was measured hourly for 6 h, beginning immediately after injection of drug or saline.

Intake was determined by weighing the food cup containing the standard or the fat-supplemented diet and including spilled crumbs collected beneath the cages.

In Experiment 2, 24 h before the experiment day, rats were housed in individual cages. The day of the experiment they were divided into four groups of 24 rats each. Three groups were IP injected with different doses of salbutamol (7.5, 10, or 15 mg/kg), one group with saline (controls). The dose range used for MA-induced hyperphagia contained a lower dose (5, 7.5, or 10 mg/kg) to better study the dose-dependent effect of salbutamol. Each group was then divided into four groups of six rats each, and injected IP with either 6 U/kg insulin, 500 mg/kg 2-DG, 46 mg/kg MA, or saline, and the cumulative intake of food eaten by the animals measured every hour during the following 6 h. The doses of insulin, 2-DG, and MA were chosen on the basis of the results of Experiment 1 as those causing a submaximal increase in food consumption as also previously reported (7,12,25).

TABLE 1
COMPOSITION OF DIETS

	Diet	
	Standard	Fat
Pure milk casein	13.0	13.0
Corn starch	73.0	48.0
Soya oil	3.0	3.4
Lard	2.4	14.6
Wesson mineral mixture*	4.0	4.0
Vitamin mixture†	1.0	1.0
Pure cellulose	3.6	16.0
Total	100.0	100.0

*Percentage of the various components of Wesson mineral mixture: $CaCO_3$ 21.0; $Ca_3(PO_4)_2$ 14.9; KH_2PO_4 31.0; KCl 12.0; NaCl 10.5; $MgSO_4$ 9.0; $MnSO_4 \cdot H_2O$ 0.02; $FePO_4 \cdot 4H_2O$ 1.47; $CuSO_4 \cdot 5H_2O$ 0.039; KJ 0.005; NaFl 0.057; $K_2Al(SO_4) \cdot 24H_2O$ 0.009.

†1 g vitamin mixture contained 0.6 mg B_1, 1.3 mg B_2, 0.4 mg B_6, 5.0 mg nicotinic acid, 4.0 mg pantothenate, 100 mg inositol, 200 mg choline, 0.1 mg biotin, 2.5 mg para-aminobenzoic acid, 1.0 mg folic acid, 5 μg B_{12}, 400 IU A, 100 IU D_3, 12 mg E, 100 μg K_3, up to 1 g sucrose. 1 g vitamin mixture for 100 g diet.

Statistical Analysis

The weight of food consumed was converted to g per 100 g of body weight. Data are presented as means ± SE and were statistically analyzed by analysis of variance (ANOVA) together with Newman–Keuls multiple comparison post hoc test.

Drugs

The following drugs were used: 2-DG (U.S. Biochemical Corporation, Cleveland, OH), insulin (Eli Lilli, Indianapolis,

USA), MA (Sigma, Milan, Italy), and salbutamol sulphate (Valeas, Italy). Drugs were dissolved in saline and administered IP in a volume of 0.5 ml per 100 g of body weight. Salbutamol was given 15 min before insulin, 2-DG, MA, or saline.

RESULTS

Effect of 2-DG, Insulin, and MA Injection on Feeding Behavior

Figure 1a and b shows the effects of IP injection of different dose of 2-DG or insulin on food intake in rats fed on the standard diet. As previously shown (28,29), 2-DG and insulin administration to rats given standard lab chow substantially increased food intake for at least 6 h after injection. These increases were statistically significant ($p < 0.01$, in post hoc test after significant ANOVA). In most instances the stimulatory effects of insulin and 2-DG were already evident 1 h after injection, even if 2-DG induced marked sedation, stupor, and ataxia, indicating that the eating was so imperious to overcome this evident exhaustion of animals. Inset of Fig. 1c shows the effect of IP injection of 46 mg/kg MA on food intake in rats fed on the standard diet. As already described (25), MA did not significantly influence the food intake of rat given this diet, when compared with controls (Fig. 1c). On a standard diet energy derived from fatty acid oxidation is quantitatively not so relevant. Therefore, the negative results concerning food intake in MA-treated rats might be due to the low rate of fatty acid oxidation under the control conditions. Thus, the fat content of the diet was increased, and the effects of different doses of MA on food intake were studied. In comparison to the controls, food intake was dose-dependently increased after 2 h, compared to saline injected rats (Fig. 1c). This increment persisted until 5 h from the MA injection (Fig. 1c). These increases were statistically significant ($p < 0.01$, in post hoc test after significant ANOVA). Throughout the experimentation MA-treated rats did not appear sedated and their spontaneous locomotor activity was comparable to that of the saline-injected rats.

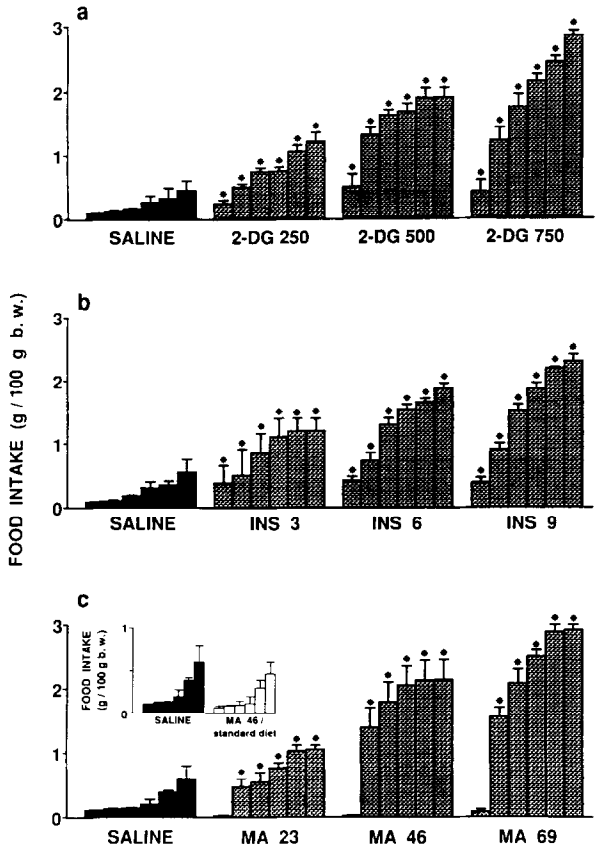

FIG. 1. Effects of different doses of 2-deoxy-D-glucose (2-DG) (a), insulin (b), and sodium mercaptoacetate (MA) (c) on rat food intake. Numbers near drugs indicate the doses expressed as mg/kg body weight for 2-DG and MA, and as IU/kg body weight for insulin. Saline, 2-DG, and insulin were IP injected in rats fed on standard diet, whereas MA was IP injected in rats trained to eat a fat diet for 2 weeks before experiments. Inset represents the effect of 46 mg/kg body weight MA on food intake IP injected in rats fed on a standard diet. Data are representative of single experiments with four experimental groups performed in the same experimental session. Bars represent means ± SE. *$p < 0.01$ vs. saline.

Effects of Salbutamol Administration on 2-DG-, Insulin-, and MA-Induced Hyperphagias

To study the effects of salbutamol on the experimentally induced hyperphagias, different doses of salbutamol, shown to be active in reducing food intake in rats trained to eat 4 h a day (4), were given 15 min before 2-DG, insulin, or MA. As shown in Fig. 2, salbutamol, given alone to rats subsequently injected with saline, did not modify the basal food intake in a statistically significant manner, even though a reduction trend was evident. The lack of an evident anorectic effect by salbutamol was expected under these experimental conditions, in which fed animals had low basal food intake, and further reductions could barely reach statistically significant levels. In addition, Fig. 2 shows that the hyperphagia induced by MA was apparently more sensitive to salbutamol than that induced by insulin. Indeed, lower doses of salbutamol were needed to antagonize the MA effect, 5 mg/kg already reducing the increased food intake by more than 50%, and 7.5 mg/kg inducing an already maximal effect. Finally, as shown in Fig. 2, in some cases salbutamol caused a delay in the onset of feeding. This was particularly evident in animals challenged with insulin.

On the contrary, even the highest dose of salbutamol (15 mg/kg) did not change at all the 2-DG-induced hyperphagia (Fig. 2). The highest dose of salbutamol did not change the animal behavior, particularly the locomotor activity, leaving the rats sedated by 2-DG. This indicates that the lack of antagonism could not be due to any unspecified effect of salbutamol on behavioral drive.

DISCUSSION

The present study shows for the first time that salbutamol antagonizes both the insulin- and MA-induced hyperphagias, with dose-related reductions in food intake of rats treated with insulin or complete dose-dependent suppression of rats treated with MA. On the contrary, salbutamol, even at the highest dose tested, is completely ineffective on the 2-DG-induced hyperphagia.

Administration of 2-DG, a glucose analogue that induces intracellular glucopenia (5), or hypoglycemic doses of insulin cause hyperphagia in rats as a result of cerebral glucoprivation (28). Because 2-DG causes not only hyperphagia, but also hyperglycemia and a state of relative insulin deficiency or resistance (11), similar to that observed in diabetes, rats given

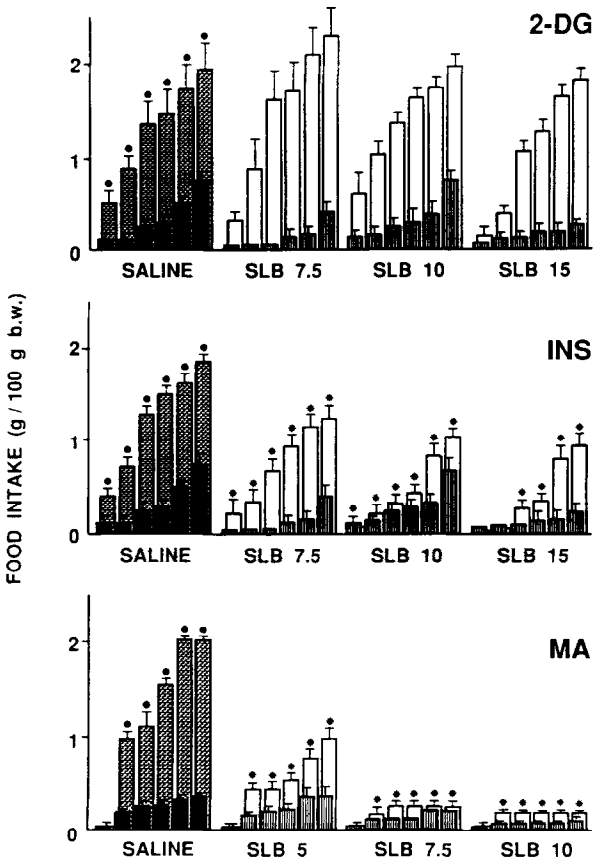

FIG. 2. Effects of different doses of salbutamol on 2-deoxy-D-glucose (2-DG), insulin, and sodium mercaptoacetate (MA)-induced increase in food intake. The effect of three doses of salbutamol (as indicated near SLB) on MA was analyzed in rats fed on fat diet. Rats were given IP saline plus saline (filled columns), or saline plus 500 mg/kg 2-DG, or 6 U/kg insulin, or 46 mg/kg MA (hatched columns), or salbutamol plus saline (lined columns), or salbutamol plus 2-DG, or insulin, or MA (doses as before) (open columns). Bars represent mean values ± SE. *$p < 0.01$ vs. saline plus 2-DG-, insulin-, MA-treated rats.

this compound may be considered as experimental models of an obese diabetic individual (30). On the other hand, the insulin-induced increase in food intake seems more representative of the hyperphagia present in obese subjects with hyperinsulinemia (2).

Our results, showing differential effects of salbutamol on 2-DG- and insulin-induced eating, support the previously proposed notion that different neural circuits are involved in the hyperphagic responses to 2-DG and insulin glucoprivation (7). Evidence for this was also provided by the observation that drugs causing anorexia by enhancing brain serotoninergic neurotransmission antagonize both 2-DG- and insulin-induced hyperphagia, whereas drugs that induce anorexia primarily by enhancing dopaminergic neurotransmission were effective only in antagonizing the hyperphagia induced by 2-DG but not that induced by insulin (7). In addition, 2-DG-induced hyperphagia needs functional alpha-adrenoceptors to bring about increased food intake and insulin does not (8). Furthermore, although activation of central and/or peripheral serotoninergic mechanisms does antagonize the overeating induced by insulin or 2-DG, impairment of serotoninergic function by the receptor antagonist metergoline does not seem to facilitate it (9).

Because salbutamol may exert peripheral metabolic effects (i.e., increase in plasma glucose levels) (13,19), the question arises whether the antagonistic effect of salbutamol on insulin-induced hyperphagia is centrally or peripherally mediated. Our results do not provide any direct evidence to answer this question. However, the following considerations would suggest a central site of action rather than a peripheral one. Indeed, the effects of salbutamol on food intake have been ascribed to a direct central mechanism because reduction of food intake can be observed after ICV injection of the drug (15). Furthermore, the observation of a differential effect of salbutamol on insulin- and 2-DG-induced hyperphagias might confirm a central site of action, because the increase of blood glucose levels induced by salbutamol would have affected both insulin- and 2-DG-induced glucoprivation. Thus, it seems likely to hypothesize that salbutamol modifies the eating response to insulin by directly modulating the neuronal system responsible for the behavioral responses rather than by influencing neurons involved in decodifying the peripheral metabolic signals.

MA stimulates feeding by activating a system capable of integrating signals involved in peripheral, but not central, availability of metabolic fuels (26). Indeed, MA had been shown to inhibit the acyl-CoA dehydrogenases located in the mitochondrial matrix and therefore it is believed to impair mitochondrial β-oxidation of fatty acids (1). Thus, these findings support the existence of a control of feeding based on fat metabolism (i.e., lipoprivic control), which is modulated through different neuronal systems (12).

Our results confirm that MA increases feeding only in rats fed the fat diet, in which the rate of fatty acid oxidation before administration is likely to be relatively high. In addition, showing that salbutamol completely antagonized the hyperphagia induced by lipoprivation, the results indicate that beta$_2$-adrenoreceptors are involved in feeding control derived from peripheral metabolic stimuli. Furthermore, it appears that 2-DG and insulin activate metabolically distinct systems for control of food intake from those activated by MA. Our findings that the beta-adrenergic anorectic drug salbutamol differently affected these experimentally induced hyperphagias are consistent with other studies demonstrating that signals transmitted to the brain by 2-DG-, insulin-, and MA-sensitive neural systems employ different central neural pathways (22,23). One further implication is that both the insulin- and MA-induced eating may ultimately be routed through the PFH where the effect is mediated via beta-adrenoreceptors. This could be consistent with the involvement of PFH beta-adrenoreceptors in feeding and the blockade of insulin- and MA-induced eating by salbutamol. The observed effects are consistent with this idea but, of course, they do not rule out other possible interpretations.

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