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Effect of Mazindol, d-Amphetamine and Diethylpropion on Purine Nucleotide Binding to Brown Adipose Tissue[1]

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LUPIEN, J. R. AND G. A. BRAY. *Effect of mazindol, d-amphetamine and diethylpropion on purine nucleotide binding to brown adipose tissue.* PHARMACOL BIOCHEM BEHAV 25(4) 733–738, 1986.—Amphetamine, diethylpropion and mazindol were administered to rats in both acute and chronic experiments to measure the changes in purine nucleotide (GDP) binding to the mitochondria from interscapular brown adipose tissue. There was a dose-dependent response to acute treatment with mazindol, but no such effect with diethylpropion. The effects of mazindol and amphetamine were present as early as 3 hours after treatment, and persisted for at least 48 hours, when compared to vehicle-injected rats when all rats were fasted from the time of injection until study. There was no effect when these drugs were added in vitro to mitochondria from brown adipose tissue. Diethylpropion had no effect on GDP binding either in vivo or in vitro at any of the times tested. Following 11 days of treatment with diethylpropion, amphetamine or mazindol, there was a significant increase in purine nucleotide (GDP) binding to mitochondria only in the amphetamine-treated animals. There was no difference in body weight or food intake with any of the three drugs after the third day of chronic treatment. The differences between the effects of these three drugs and those of fenfluramine are discussed in terms of their different central mechanisms of action.

Diethylpropion Amphetamine Mazindol Thermogenesis

WE have previously shown that treatment with fenfluramine significantly increases the binding of the purine nucleotide, guanosine 5'-diphosphate (GDP) to mitochondria from interscapular brown adipose tissue [7]. This effect occurred as early as 3 hours after treatment of the animals and persisted for at least 24 hours. During chronic treatment with fenfluramine, there was an initial reduction in body weight and food intake. However, food intake recovered to control levels whereas body weight remained reduced [6,7]. The increase in GDP-binding to brown adipose tissue was interpreted as an increase in thermogenesis resulting from activation of the sympathetic nervous system following drug treatment, and provides a mechanism for the reduction in body weight of these animals. The present experiments were designed to evaluate three other appetite suppressant drugs for their effects on the binding of GDP to mitochondria from brown adipose tissue. The drugs selected for study were dextro-amphetamine, diethylpropion and mazindol all of which are thought to act through noradrenergic or dopaminergic systems in the brain [4,12]. This contrasts to fenfluramine which appears to act through serotoninergic systems [2].

METHOD

Animals

Female Wistar rats weighing approximately 250 g were purchased from Simonsen Laboratory, Gilroy, CA. The animals were housed in groups of 4 or 5 in hanging wire-bottom cages except for experiment 3 where animals were housed in individual metabolic cages. The vivarium was maintained at 22±1°C. Purina Laboratory Chow (Ralston Purina, St. Louis, MO) and tap water were available ad lib until the time of injection with drug or vehicle in the acute experiments (experiment 1 and 2) and until sacrifice in the chronic experiment (experiment 3). Food intake was measured in the morning, before injecting drugs, in experiment 3.

GDP Binding

Rats were killed by decapitation and the interscapular brown adipose tissue was carefully removed and trimmed from adhering white fat and muscle. The tissue was homogenized in a glass, motor driven tissue homogenizer in a medium containing 0.25 M sucrose, 0.2 mM EDTA (potassium salt), and 1 mM HEPES, pH 7.2 at 0°C and mitochondria were isolated as described by Slinde and Pedersen [13]. The binding of guanosine 5'-diphosphate (GDP) to brown adipose tissue mitochondria was performed by the method of Nicholls [8], as modified by Desautels and Himms-Hagen [3]. Isolated mitochondria (0.5 mg of protein/ml) were incubated for 10 minutes at room temperature (20°C) in a medium containing 10 μM 3H-GDP (1.25 μCi/ml), 14C-sucrose (0.1 Ci/ml), 100 mM sucrose, 20 mM K-TES (pH 7.1), 1 mM

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K-EDTA, 10 mM choline chloride and 5 μM rotenone. The mitochondria with the radioactive GDP bound were separated by centrifugation for 3 minutes in an Eppendorf microfuge, dissolved by incubation in 0.75 N NaOH and counted in a liquid scintillation counter. A correction for the quantity of trapped medium was made from the C-14-sucrose radioactivity on the filter. Protein was estimated by a modification of the Lowry method [10].

Experimental Procedures

Three experiments were performed. In the first experiment, groups of animals were injected with drug and food was withheld for the ensuing 3 hours. As part of the first experiment the effect of each drug on the binding of GDP to mitochondria in vitro was compared in animals fed until sacrifice. In experiment 2 food was withheld from the time drug or vehicle was injected until sacrifice, 3, 24 or 48 hours later. In experiment 3, the animals were injected each morning for 11 days. Drugs were dissolved in physiological saline and injected intraperitoneally in a volume of 2.5 ml/kg body weight. Both the control groups and the experimental groups were injected in the morning between 0800 and 0900 hr. Dose-response curves were constructed for diethylpropion and mazindol. Only a single dose of dextro-amphetamine was used.

Chemicals

Diethylpropion was provided by Merrill-National Laboratories (Cincinnati, OH). Dextro-amphetamine was obtained from the pharmacy. Mazindol was a gift of the Wyeth Laboratories (Philadelphia, PA). The other chemicals were purchased from Sigma Chemicals Co (St. Louis, MO). The radiochemicals were obtained from New England Nuclear Corp (Boston, MA).

Statistics

A one-way analysis of variance was carried out on all data in experiments 1 and 2. In the chronic experiment, a two-way analysis of variance was performed to examine the effects of time and treatment on food intake and body weight and a one way ANOVA for the effects of treatment on GDP binding. Contrasts between subgroups were performed by the Neuman-Keuls method.

RESULTS

Experiment 1

The response to 3 doses of mazindol and diethylpropion and a single dose of amphetamine is shown in Fig. 1. All measurements were made 3 hours after injecting the drug. There is a dose-dependent response to mazindol, but no response to any dose of diethylpropion. The single dose of d-amphetamine (mg/kg) produced a significantly greater ($p<0.001$) increase in GDP binding than the same dose of mazindol. This response to d-amphetamine was similar to the highest dose of mazindol, suggesting that amphetamine was more potent than mazindol. Addition of mazindol, dextro-amphetamine, or diethylpropion in a concentration of 30 μg/ml to the incubation medium containing mitochondria from brown adipose tissue did not change the binding of GDP to these mitochondria compared to that found with untreated mitochondria.

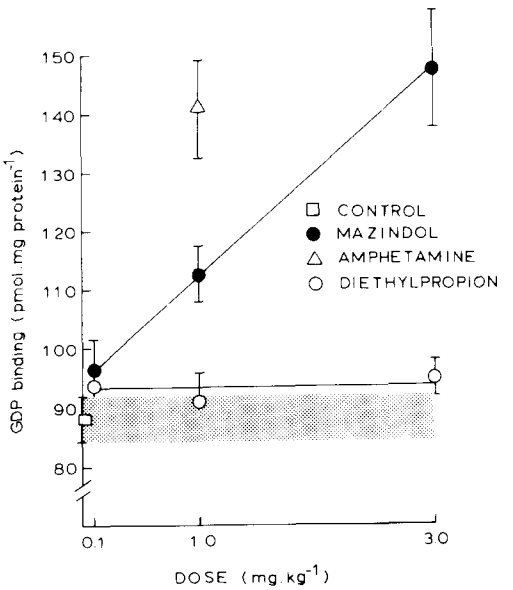

FIG. 1. Response of purine binding (GDP) to treatment with mazindol, d-amphetamine and diethylpropion. The dose-dependent changes in GDP-binding to mitochondria from brown adipose tissue after acute treatment in vivo with mazindol and diethylpropion are plotted along with the response to a single dose of amphetamine. The measurements were made 3 hours after injecting the animals with the stated dose of each drug. The effect of d-amphetamine was significantly greater ($p<0.001$) than mazindol at the same dose. The points are the mean±SEM.

Experiment 2

Both mazindol at 3 mg/kg, and amphetamine at 1 mg/kg, produced a significant increase in the binding of GDP to mitochondria from interscapular brown adipose tissue 3 hours following the injection (Fig. 2). This effect was also significant at 24 and 48 hours after the injection in rats which had been starved from the time of injection. At the 24 hour time point, the effect of amphetamine was significantly greater ($p<0.001$) than the mazindol-treated group. For diethylpropion, on the other hand, there was no increase of GDP binding at any time interval.

Experiment 3

Rats were treated with mazindol, diethylpropion or amphetamine at a dose level of 1 mg/kg body weight for amphetamine and 3 mg/kg body weight for mazindol and diethylpropion. Food was available ad lib, and the last injection was given 24 hours before sacrifice. The mean body weights are plotted in Fig. 3 and mean food intake data in Fig. 4. To avoid clutter the standard error bars are shown only for the control group except for day 1 and 2 of food intake where they were added to the mazindol points. The mean body weight of the drug treated rats declined slightly during the first 3 days of treatment but, thereafter, was within one standard error of that of the controls (Fig. 3). Food intake in the mazindol-treated rats was significantly depressed during the first 2 days of treatment (Fig. 4). For the remainder of the treatment food intake varied some from day to day, but there was no significant effect of any drug. The weight of interscapular brown adipose tissue was significantly higher in the diethylpropion-treated rats than in rats treated with mazin-

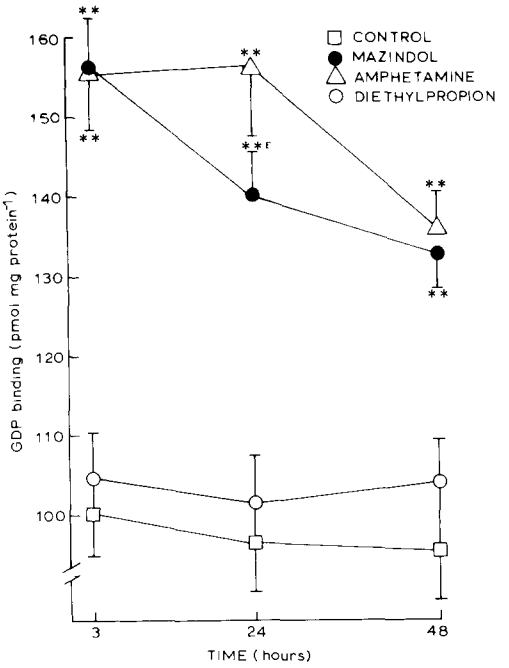

FIG. 2. Time-course for response of GDP-binding to treatment with mazindol, d-amphetamine and diethylpropion. A dose of 1 mg/kg amphetamine and 3 mg/kg mazindol and diethylpropion was given to each animal at zero time. All animals were deprived of food after the injection of drug. Interscapular brown adipose tissue was obtained for examination at 3, 24 and 48 hours following injection in separate groups of animals. The data are expressed at mean±SEM. The symbols with ** are significantly greater ($p<0.01$) than the corresponding vehicle treated animals. The symbol £ denotes a significantly greater effect ($p<0.05$) of the d-amphetamine-treated group compared to the mazindol-treated group.

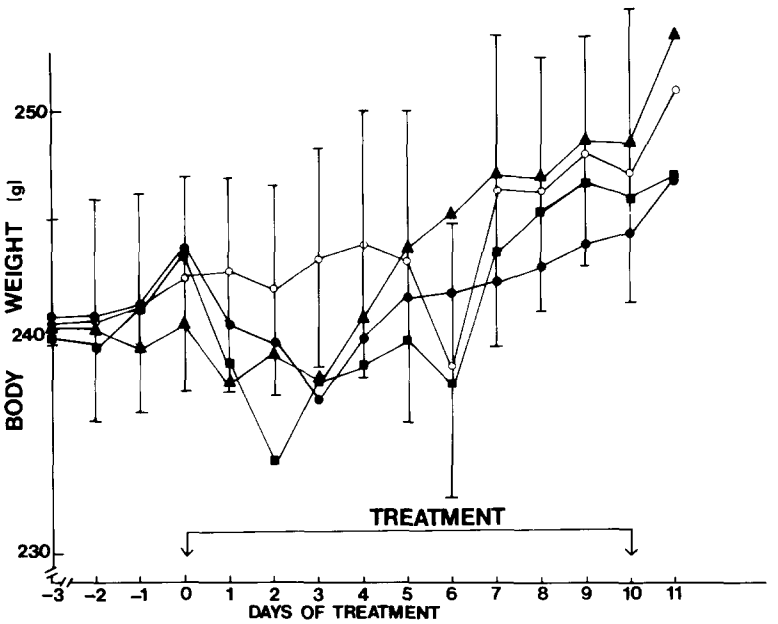

FIG. 3. Body weight of rats treated for 11 days with anorectic drugs. Vehicle (○—○), mazindol 3 mg/kg (■—■), diethylpropion 3 mg/kg (●—●) and d-amphetamine 1 mg/kg (▲——▲) were given once daily between 0800–0900 hr, following measurement of body weight. Mean values are given for each group with the SEM for the vehicle-treated group.

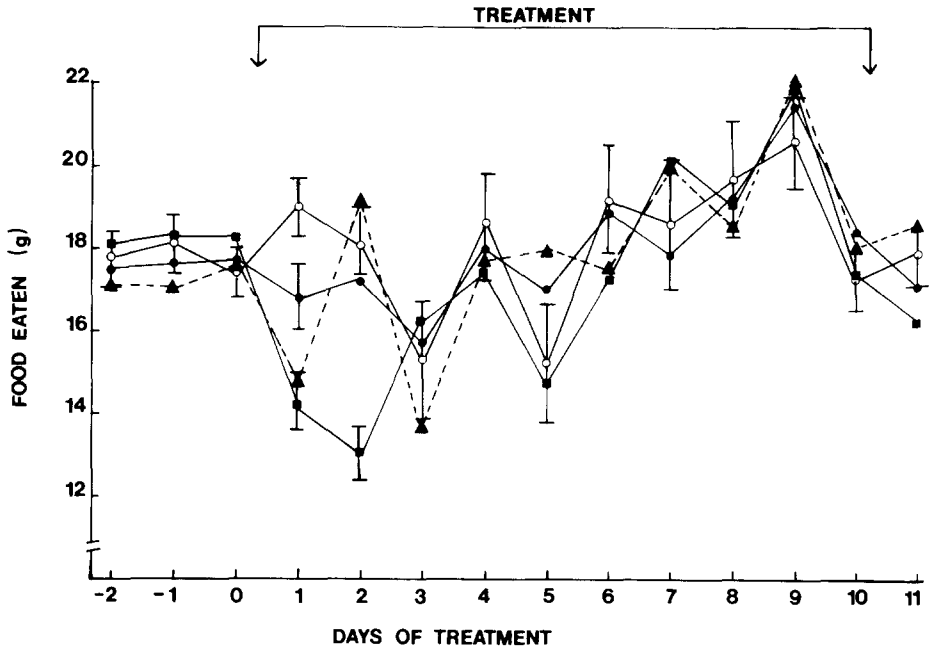

FIG. 4. Food intake of rats treated for 11 days with anorectic drugs. Vehicle (○—○), mazindol 3 mg/kg (■—■), diethylpropion 3 mg/kg (●—●), and amphetamine 1 mg/kg (▲— —▲) were given once daily between 0800 and 0900 after measuring food intake. The last injection was 24 hours prior to sacrifice. Mean values are given for each group with vertical lines for SEM of control group and mazindol group on day 1 and 2.

TABLE 1

PURINE NUCLEOTIDE (GDP) BINDING TO INTERSCAPULAR BROWN ADIPOSE TISSUE MITOCHONDRIA FROM RATS TREATED-CHRONICALLY WITH APPETITE SUPPRESSANTS*

	Saline	Diethylproprion	Amphetamine	Mazindol
Body Weight (g) (after exsaguination)	244 ± 8	254 ± 6	247 ± 3	244 ± 6
Interscapular brown adipose tissue mass (mg)	269 ± 9	303 ± 8[b]	291 ± 14[ab]	233 ± 16[ab]
Mitrchondrial protein (mg)	3.1 ± 0.1	3.3 ± 0.1	3.5 ± 0.2	3.1 ± 0.2
Total protein (mg)	38.3 ± 2.2	38.7 ± 1.2	40.7 ± 1.9	33.3 ± 1.3
Specific GDP binding (mol/mg protein)	81.9 ± 7.6[c]	80.8 ± 3.1[b]	123.0 ± 4.8[abc]	103 ± 7.5[a]
n	6	6	6	6

*Rats were treated daily with a single dose of amphetamine (1 mg/kg), diethylproprion (3 mg/kg), or mazindol (3 mg/kg) for 11 days.

Results are mean ± SEM.

[a]$p<0.05$ between pairs with the same letter.

[b]and [c]$=p<0.01$ between pairs with the same letter.

dol. IBAT from d-amphetamine-treated rats was also heavier than in the mazindol-treated rats. However, only in the amphetamine-treated rats was there an increase in the specific GDP binding (Table 1).

DISCUSSION

These studies have shown that both dextro-amphetamine and mazindol will acutely increase the binding of the purine nucleotide, guanosine 5'-diphosphate (GDP), to mitochondria from brown adipose tissue of rats, whereas diethylpropion had no effects. During chronic treatment with these 3 drugs, there was an initial fall in food intake and body weight in the mazindol-treated controls. After eleven days of treatment, only amphetamine treated rats showed a significant increase in GDP binding to mitochondria from brown adipose tissue.

The present experiments have extended the previous studies on the effects of fenfluramine on the binding of GDP to brown adipose tissue to 3 appetite suppressant drugs which have a different mechanism of action [4]. In the previous studies [7] we found that there was a dose-response increase in binding of GDP to mitochondria from brown adipose tissue after acute treatment with fenfluramine. This effect was present as early as 3 hours and lasted for 48 hours. The present studies show an essentially identical pattern of acute response to treatment with mazindol and amphetamine. These acute effects persisted for at least 48 hours. Treatment with diethylpropion, on the other hand, was without effect. Both mazindol and d-amphetamine have been shown to acutely increase resting metabolic rate in rats [9], but fenfluramine was without effect on this system in vivo [9]. Moreover, amphetamine and mazindol can increase the activity of the sodium pump (Na-K ATPase) in brown adipose tissue [9], an enzyme which is highly correlated to oxygen uptake in brown adipose tissue.

The chronic treatment with all three of the drugs used in this experiment was different than previously observed with fenfluramine [6,7]. With fenfluramine, there was an initial decrease in food intake and a gradual fall in body weight [6,7]. However, food intake slowly returned to normal levels although body weight remained depressed. None of the drugs used in this experiment chronically depressed body weight. Our findings with mazindol differ from those of Wyllie *et al.* [16]. In their study rats were treated with 1 mg/kg by stomach tube twice daily. On this regime there was a significant increase in GDP binding and a significant reduction in gross energy efficiency. Had we used twice daily dosing we might have found an increase in GDP binding with mazindol.

These differences in the response to chronic treatment with fenfluramine and mazindol once daily may be a reflection of the underlying mechanistic differences between fenfluramine and the other appetite suppressants and/or may be due to differences in their pharmacokinetics. Amphetamine, mazindol, and diethylpropion are thought to act by increasing the release and/or blocking the re-uptake of endogenous norepinephrine or dopamine at the neuroeffector junction [4, 8, 9]. Fenfluramine, on the other hand, is thought to act primarily through the serotonin system [2], since its effect on food intake is blocked by drugs which block the action of endogenous serotonin. Previous studies from our laboratory [7] and elsewhere [6] show that treatment with fenfluramine can effect a chronic reduction in body weight. This effect is not produced by chronic treatment with the appetite suppressants which influence the endogenous catecholamines. These findings imply that the mechanism by which the efferent sympathetic fibers to IBAT are activated involves a central serotoninergic system and not a noradrenergic one. The fact that chronic treatment with amphetamine increased GDP binding but did not lower body weight is difficult to reconcile with the normal food intake and body weight in these animals. It suggests that GDP binding, by itself, may not necessarily reflect changes in energy balance.

Mazindol increased GDP binding which lasted 48 hours. However, after 11 days of treatment, no effect on GDP binding was detectable 24 hr after the last injection. Fenfluramine, on the other hand, was still effective in suppressing body weight and increasing GDP binding after 11 days of treatment after a single daily dose given 24 hr before body weight was measured. In addition to differences in the central sites of action of these drugs, differences in pharmacokinetics may also help account for the difference between acute and chronic treatment with mazindol and fenfluramine. Chronic treatment with mazindol may induce drug metabolizing enzymes which hasten its removal. Alternatively, the route of administration (oral vs. IP) may help explain the differences between our data and those of Wyllie *et al.* [16]. Whether these potential differences in drug metabolism are sufficient to account for the differences we have observed between the two drugs must await further study.

The possibility that some appetite suppressant drugs might act as thermogenic drugs has been explored in both man and animals [1,14]. In man, amphetamine can produce a 15% increase in metabolic rate following its acute administration [1]. However, the reduction of food intake by amphetamine appears to be its major mechanism of action [5]. In animals, both d-amphetamine and mazindol were shown to increase resting oxygen consumption in conscious rats [9,14]. Diethylpropion, on the other hand, was without effect [14]. Our data on the effects of amphetamine, mazindol, and diethylpropion on GDP binding to brown adipose tissue are consistent with the observations on total oxygen consumption [9,14]. Diethylpropion appears to be essentially devoid of thermogenic effects. Mazindol and amphetamine, on the other hand, both have acute effects on thermogenesis and on the binding of GDP to brown adipose tissue. However, only fenfluramine appears to be effective in chronically reducing the body weight and body fat by a mechanism which appears to be thermogenic [7].

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