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Effect of Vasoactive Agents on Fatty Acid Metabolism: Inhibition of Lipolysis by Tipropidil in Rat Epididymal Adipose Tissue (41319)

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Abstract. The effect of Tipropidil (MJ 12880-1), a potent vasoactive drug, on fatty acid mobilization in rat adipose tissue was investigated. Oral administration of a single dose of MJ 12880-1 (50 mg/kg body wt) did not cause any change in glucose tolerance. Increase in the dose of ingestion to 300 mg/kg body wt was found to result in slight elevation in plasma free fatty acid levels without causing any alteration in blood glucose. Prolonged oral administration of the drug at a dose of 25 mg/kg body wt/day for 2 weeks brought about significant increase in the plasma free fatty acid concentration. Free fatty acid content in the epididymal fat pads of MJ 12880-1-treated rats was slightly higher than the controls. In comparison to the controls epididymal adipose pads from MJ 12880-1-treated rats showed significantly lower lipolytic rate and responded to a lesser degree to norepinephrine and theophylline. The increase in plasma free fatty acid levels and decrease in lipolytic response were found to be dependent on the amount of drug administered. Addition of MJ 12880-1 to the *in vitro* lipolysis system inhibited norepinephrine- and theophylline-induced fatty acid release in a dose-dependent manner. The results of this study suggest a potent inhibitory action for Tipropidyl on fatty acid mobilization in rat adipose tissue.

Suloctidil [1-(4-isopropylthiophenyl)-2-*n*-octylaminopropranolol] and Tipropidil [1-(4-[1-methylethyl]thiophenoxy-3-[octylamino])-2-propranolol hydrochloride] are structurally similar drugs (Fig. 1), designed for the treatment of cerebral and peripheral vascular diseases. Suloctidil is an effective vasoactive and antispasmodic drug possessing potent inhibitory activity on platelet aggregation (1). Besides its vasoactive and antispasmodic effects Suloctidil has been demonstrated to exert hypolipidemic and antilipolytic activity (2). Because of the close structural resemblance of Tipropidil with Suloctidil it is possible that Tipropidil may exert similar influence on pharmacological and biochemical parameters. The present experiments were designed, therefore, to investigate the action of Tipropidil on lipid metabolism. In this communication we report *in vivo* and *in vitro* effects of Tipropidil on fatty acid mobilization in rat epididymal adipose tissue.

Materials and Methods. *Animals and treatment.* Male rats of Sprague–Dawley strain were used in this study. They were divided into control and various experimental groups. Animals in the experimental group were orally administered, with the help of an intragastric cannula, appropriate amounts of MJ 12880-1 or MJ 12637 as suspension in 0.5% methocel. Doses and feeding schedules are mentioned under individual experimental conditions. Daily doses of the drugs were administered in equal half quantities in the morning and evening. Animals in the control groups were fed 0.5% methocel in lieu of the drugs. All animals were fed a regular chow diet and had free access to water.

Suloctidil (MJ 12637) and Tipropidil hydrochloride (MJ 12880-1) were supplied by Bristol-Myers Company, International Division, New York, New York. Methocel, a 0.5% solution of which was used as the vehicle to suspend the drugs, was a product of Dow Chemical Company, Indianapolis, Indiana. All other chemicals used in the study were purchased from Sigma Chemical Company, St. Louis, Missouri.

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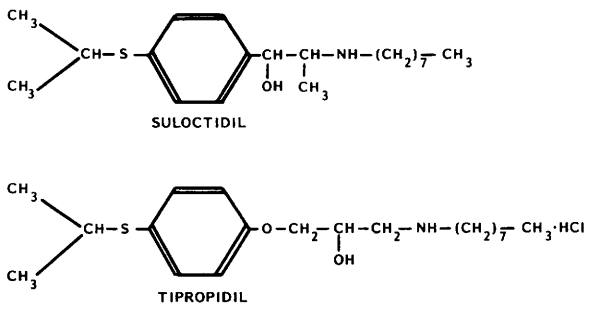

FIG. 1. Structures of Suloctidil (MJ 12637) and Tipropidil hydrochloride (MJ 12880-1).

Assays. Blood glucose: Blood samples were quickly deproteinized by treating with barium hydroxide and zinc sulfate (3) and the protein-free supernatants were analyzed for their glucose content by glucose oxidase method (4).

Free fatty acid: Plasma free fatty acids were extracted and assayed by the method of Dole and Meinertz (5).

Assay of lipolysis: Immediately after sacrificing the animals epididymal fat pads were rapidly excised, weighed (80–100 mg), and placed in 3.0 ml Krebs–Ringer bicarbonate buffer (pH 7.4) containing half the prescribed concentration of calcium (6). The incubation medium also contained 3.5% bovine serum albumin (essentially fatty acid free). Incubation at 37° was carried out for 2 hr in a Dubnoff metabolic shaker. An atmosphere of oxygen:carbon dioxide (95:5) was maintained throughout the incubation period. Incubation was terminated by quickly chilling and removing the tissue from the medium. An aliquot of the medium was immediately transferred into the extraction mixture and the free fatty acids were extracted and assayed by the method of Dole and Meinertz (5).

Results. *Effect of oral administration of a single dose of MJ 12880-1 and MJ 12637 on glucose tolerance, blood glucose, and plasma free fatty levels.* Oral administration of a single dose of MJ 12880-1 or MJ 12637 at a dose of 50 mg/kg body wt to fasting animals did not cause any change in the glucose tolerance profile (Fig. 2). Effect of large excess doses of these drugs on blood glucose and plasma free fatty acids were also investigated. As evident from Table I ingestion of either MJ 12880-1 or MJ-12637 at a dose of 300 mg/kg body wt did not bring about any alteration in blood glucose. In comparison to the controls, animals ingested with a large dose of MJ 12880-1 or MJ 12637 (300 mg/kg body wt)

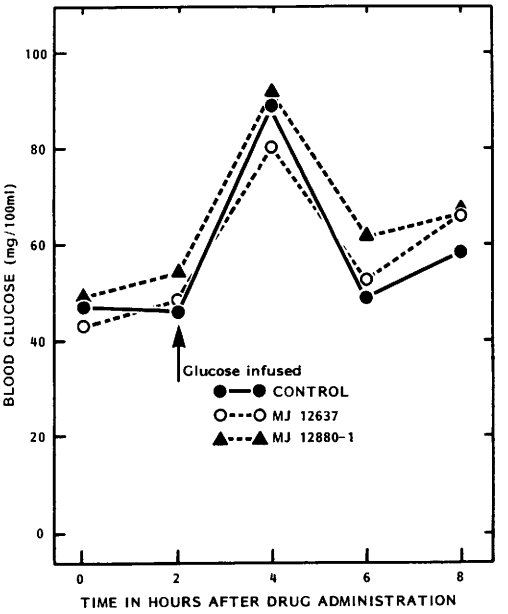

FIG. 2. Effect of ingestion of a single dose of MJ 12637 or MJ 12880-1 on glucose tolerance. Male rats weighing 200–240 g were fasted for 20 hr. They were divided into three groups: Control, MJ 12637, and MJ 12880-1. Each rat in the experimental group was orally fed either MJ 12637 or 12880-1 at a dose of 50 mg/kg body wt. Appropriate volumes of the vehicle solution (0.5% methocel) were fed to the animals of the control group. Two hours after administration of the drugs animals were orally given a glucose load (1.25 g/kg body wt). Blood samples were taken at 0, 2, 4, 6, and 8 hr and analyzed for their glucose content. Each value plotted is the mean of four observations.

TABLE I. EFFECT OF SINGLE LARGE DOSE OF SULOCTIDIL (MJ 12637) AND TIPROPIDIL (MJ 12880-1) ON THE LEVELS OF BLOOD GLUCOSE AND PLASMA FREE FATTY ACIDS

	Glucose (mg/100 ml)	FFA (μeq/liter)
Control	75.2 ± 3.8[a]	450 ± 42
MJ 12637	80.1 ± 5.8	530 ± 55*
MJ 12880-1	77.6 ± 2.4	533 ± 61*

Note. Animals in experimental groups were orally fed, with the help of a cannula, MJ 12637 or MJ 12880-1 at a dose of 300 mg/kg body wt. Rats in the control group were fed appropriate volume of the vehicle solution (0.5% methocel) in lieu of the drug. Animals were sacrificed at the end of 6 hr. Blood glucose and plasma FFA were determined as described under Materials and Methods.

[a] Each value given is mean ± SEM of the results from four animals.

* Change is not significant, $P > 0.05$.

showed slightly higher levels of free fatty acids in the plasma. Although the increase could be accounted to 18% over the control values, the difference was not statistically significant ($P > 0.05$).

Effect of daily oral ingestion of MJ 12880-1 and MJ 12637 on blood glucose, plasma free fatty acids, and adipose tissue lypolysis. Table II shows the effect of continued oral administration of MJ 12880-1 or MJ 12637 on body weight and on the levels of blood glucose and plasma free fatty acids. As can be noted, prolonged ingestion of these drugs at a daily dose of 25 mg/kg body wt for a period of 2 weeks did not cause in any alteration in body weight or in blood glucose levels. On the other hand, plasma free fatty acids were markedly increased as a result of the treatment.

In order to examine the dose dependence of the treatment of MJ 12880-1 on plasma free fatty acids, different daily doses of the drug (6.25, 12.5, and 25.0 mg/kg body wt) were administered for a period of 2 weeks. Figure 3 depicts the levels of plasma free fatty acid and blood glucose in rats administered different amounts of MJ 12880-1. The results reveal that increase in the dose of MJ 12881-1 caused elevation in plasma free fatty acid concentration in a dose-dependent fashion. Ingestion of the drug, however, did not cause any change in blood glucose level.

Effect of MJ 12880-1 on fatty acid mobilization. Effects in vivo. Based on the findings that ingestion of MJ 12880-1 brings about elevated levels of plasma free fatty acids it was thought worthwhile to examine their effect on fatty acid release from the adipose tissue. Data presented in Fig. 4 indicate the effect of long-term feeding of different doses of MJ 12880-1 on norepinephrine- and theophylline-induced lipolysis. As evident from the figure, administration of MJ 12880-1 markedly decreased the basal lipolytic rate measured in terms of fatty acid release. The inhibition in lipolytic rate can be observed even in rats administered with the lowest dose of the drug (6.25 mg/kg/day). The inhibitory effect was found to increase progressively with the administered dose of MJ 12880-1. The stimulatory effect of norepinephrine and theophylline on lipolysis was found to be significantly less in MJ 12880-1-treated animals. The inhibition in response to norepinephrine or theophylline was found to be related to the dose of MJ 12880-1 administered.

Data presented in Table III show the free fatty acid content in liver and epididymal adipose tissue of control and MJ 12880-1-treated rats. Administration of MJ 12880-1 for 2 weeks at a dose of 25 mg/kg body wt/day resulted in a significant decrease in FFA content of the liver. On the other hand, ingestion of the drug did not bring

TABLE II. EFFECT OF LONG-TERM TREATMENT OF MJ 12637 AND MJ 12880-1 ON THE LEVELS OF BLOOD GLUCOSE AND PLASMA FREE FATTY ACIDS

	Final body wt (g)	Glucose (mg/100 ml)	FFA (μeq/liter)
Control	207 ± 9 (11)	54.5 ± 2.9[a] (6)	487 ± 87 (5)
MJ 12637	190 ± 9 (5)	55.7 ± 4.4 (5)	1073 ± 147* (6)
MJ 12880-1	199 ± 10 (11)	59.6 ± 3.5 (6)	1040 ± 106* (6)

Note. Animals in the drug-treated group were fed MJ 12637 or MJ 12880-1 at a daily dose of 25 mg/kg body wt. for a period of fifteen days. Details of the experimental procedures are given in the section "Materials and Methods".

[a] Each value given is mean ± SEM of the number of observations indicated in parentheses.

* Significantly different from controls at $P < 0.01$.

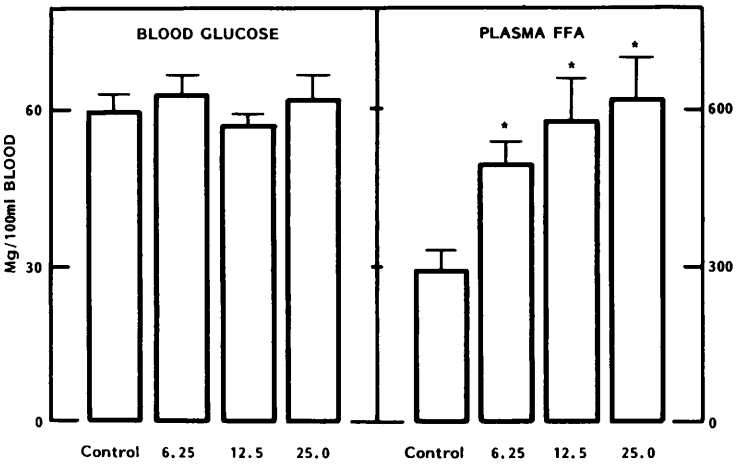

FIG. 3. Effect of ingestion different doses of MJ 12880-1 for 2 weeks on blood glucose and plasma free fatty acid levels. Male rats weighing approximately 100 g were divided into four groups. Group 1 served as the controls which were fed appropriate volume of the vehicle solution (0.5% methocel) in lieu of MJ 12880-1 suspension. Groups 2, 3, and 4 were orally fed MJ 12880-1 daily at doses of 6.25, 12.5, and 25.0 mg/kg body wt for a period of 2 weeks. Animals were fasted for 20 hr before they were sacrificed. Blood glucose and plasma fatty acid levels were determined as described under Materials and Methods. Data presented are means ± SEM of the results obtained from five or more animals.

about any significant alteration in the FFA content of the adipose tissue.

Effect of MJ 12880-1 on adipose tissue lipolysis in vitro. The possibility of a direct effect of MJ 12880-1 on fatty acid mobilization in adipose pads was investigated *in vitro*. Various concentrations of MJ 12880-1, ranging from 10^{-6} to 10^{-3} *M*, were tested on basal, norepinephrine- and theophophilline-induced lipolysis. The results are depicted in Fig. 5. As can be noted addition of MJ 12880-1 in the assay system did not alter basal rate of lipolysis. However, the drug exerted significant inhibition on lipolysis induced by norepinephrine or theophylline. The inhibitory effect was dose dependent.

Discussion. The results of the present study demonstrate that ingestion of MJ 12880-1 by rats for a period of 2 weeks causes elevation of plasma free fatty acids in spite of an inhibitory effect of the drug on adipose tissue lipolysis. This elevation of plasma FFA level observed *in vivo* cannot be due to an alteration in the nutritional status of the animals because ingestion of Tipiopidil did not alter the food intake or weight gain. Free fatty acid levels in the plasma has been generally taken as an index of the lipid mobilization from the adipose tissue *in vivo*. The elevated levels of plasma free fatty acids have also been attributed to decreased uptake and utilization of FFA by the liver. An increase in the activity of lipoprotein lipase may also cause high levels of fatty acids in the plasma. However, the results showing no change in the rate of oxidation of fatty acid by the liver slices (unpublished data) together with significantly low levels of FFA content in the livers of Tipropidil-treated rats (Table III) indicate the possibility of an inhibition in the uptake and utilization of fatty acids by the liver. Hence, the increase in plasma FFA levels in intact animals as a result of ingestion of MJ 12637 or MJ 12880-1 may be accounted for either by enhanced mobilization from adipose tissue or by increased release from lipoproteins.

Although the elevated level of plasma FFA level is indicative of a lipolytic effect by MJ 12880-1 in intact animals, the *in vitro* effects suggest a clear-cut antilipolytic property for the drug. This is evident by the finding that treatment of animals with MJ 12880-1 for 2 weeks resulted in significant decrease in the basal lipolytic rate (Fig. 4).

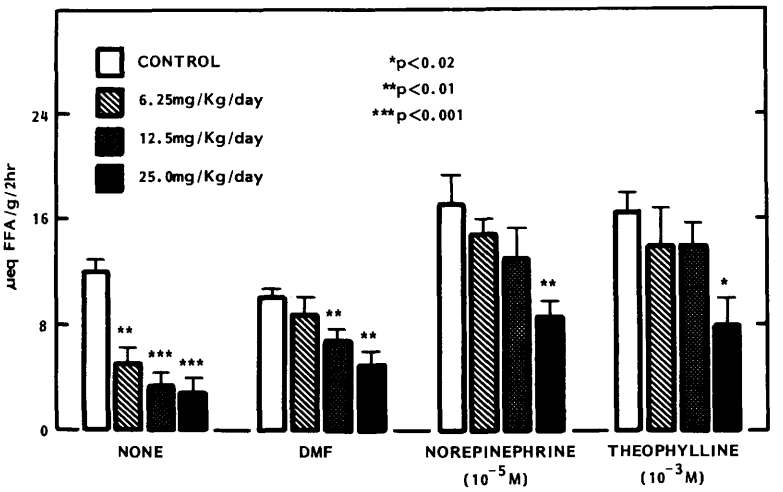

FIG. 4. Effect of ingestion of different doses of MJ 12880-1 for 2 weeks on adipose tissue lipolysis. Treatment of animals and other experimental conditions were the same as mentioned in the legend of Fig. 3 except that the animals were not fasted. 70–90 mg epididymal adipose pads were incubated in 3.0 ml Krebs–Ringer bicarbonate buffer (pH 7.4) with half the recommended amount of calcium. The incubation medium also contained 3.5% bovine serum albumin (essentially fatty acid free). Incubations with appropriate additions were carried out for 2 hr in a metabolic shaker under the atmosphere of 95% O_2 and 5% CO_2. Incubations were terminated by quickly chilling and transferring the medium into the solvent mixture for fatty acid extraction. Free fatty acid contents were extracted and assayed by the method of Dole and Meinertz (4). Each value is mean ± SEM of the results from four to six rats.

In addition, fat pads from all the three groups which were subjected to treatment with different doses of MJ 12880-1 showed reduced rate of lipolysis when induced by norepinephrine on theophylline. This effect by MJ 12880-1 may be due to alterations in cyclic AMP levels which may have been caused by changes in the activities of adenylate cyclase or phosphodiesterase or both. Since MJ 12880-1 inhibits fatty acid mobilization from the adipose tissue, it seems that an enlargement in the cell size may possibly occur. Since the morphology of the adipocytes has not been examined in the present study, it is difficult to document if there had been any enlargement in the cell size. However, the data showing no significant alterations in the free fatty acid content of the adipose tissue as a result of MJ 12280-1 ingestion (Table III), suggest that enlargement of the adipocytes might not have occurred.

TABLE III. EFFECT OF LONG-TERM FEEDING OF MJ 12880-1 ON FREE FATTY ACID CONTENT IN THE EPIDIDYMAL ADIPOSE PADS AND LIVER

	Fatty acid content (μeq/g tissue)		
Tissue	Control	MJ 12880-1	*P*
Adipose	2.67 ± 0.41[a] (4)[b]	3.47 ± 0.32 (5)	>0.05
Liver	9.93 ± 0.63 (5)	6.20 ± 0.22 (5)	<0.01

Note. Treatment of animals was the same as given in the legend of Table II. Immediately after sacrificing the animals tissues were quick-frozen by freeze clamp technique. Tissue samples were weighed and extracted for free fatty acid determination by the method of Dole and Meinertz (4).

[a] Each value is mean ± SEM.

[b] Figures in parentheses indicate the number of animals.

Results of the present study reveal an interesting phenomenon of the drug. As can be interpreted from the elevated levels of plasma FFA, MJ 12880-1 seemed to have acted as a lipolytic agent *in vivo*. The data obtained on the *in vitro* studies, however, clearly reveal that the Tipropidil has an inhibitory effect on fatty acid release in the adipose tissue. These findings lead to the postulate that there may be more than one mechanism operating in intact animals

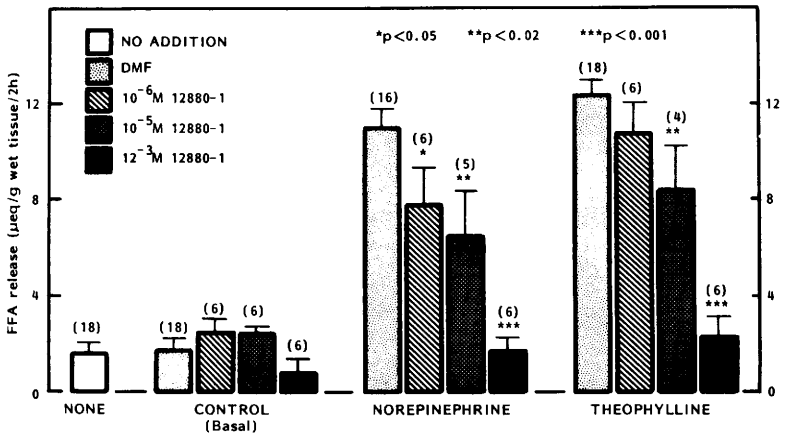

FIG. 5. Effect of MJ 12880-1 on norepinephrine- and theophylline-induced fatty acid release in epididymal adipose tissue *in vitro*. Epididymal adipose pads from normal rats (weighing approximately 200 g and fed *ad libitum*) were used in this study. Incubations and assay of lipolysis were carried out as described in the legend of Fig. 4. 10^{-5} *M* norepinephrine or 10^{-3} *M* theophylline were used to induce lipolysis. Dimethylformamide (DMF) was used as the solvent for MJ 12880-1. Each value presented is mean ± SEM. Figures in parentheses indicate number of observations.

which may eventually result in elevation of the plasma free fatty acid levels. The discrepancy between the elevated plasma FFA levels observed *in vivo* and the antilipolytic effect observed *in vitro* can not be explained satisfactorily at present. However, certain points are to be considered as possible causative factors for the opposite effects observed *in vivo* and *in vitro*. A reasonable explanation is that a metabolite or metabolites of the drug formed *in vivo* may be responsible for exerting the lipolytic effect in intact animals. Possible effects of the drugs or their metabolites on adrenal function has also to be considered in explaining the *in vivo* observations since adrenal steroids have been shown to exert permissive effect for other hormones and agents to produce responses in the cell (7, 8). The autonomic nervous system has also been known to play an important role in the enhancement of lipid mobilization *in vivo* (9). The sympathetic nervous system mediates the rise in plasma free fatty acids when the metabolic fuel for the body must be augmented rapidly (10). During conditions such as exercise the major mechanism by which lipolysis is regulated is by the local liberation of norepinephrine due to the increased activity of the sympathetic nerves in the adipose tissue (11, 12). Thus, there is a possibility that the rise in plasma FFA caused by MJ 12880-1 in intact animals might be mediated either through the autonomic nervous system or through an increased adrenal function.

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