Interactions of Sympathomimetic Amines and Adrenergic Blocking Agents at Receptor Sites Mediating Lipolysis in the Rat*† (33759)

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There is general agreement that the adrenotropic receptor mediating the release of free fatty acids produced by sympathomimetic amines is, in Ahlquist's terminology, a β adrenotropic receptor in dog, cat, and man. However, in the rat, and some other species, conflicting data exist for α and β adrenergic blockade of lipolysis produced by sympathomimetic amines (1).

In a previous study on the characterization of the glycogenolytic receptors responding to epinephrine in the intact rat, β adrenergic receptors were found to mediate epinephrine-induced glycogenolysis in the heart and skeletal muscle. In contrast, the rat liver glycogenolytic receptors did not appear to be α receptors or β receptors, nor a combination of the two types, since they were responsive to epinephrine, but not to isoproterenol, and they were blocked by dihydroergotamine, but not by other α and/or β adrenergic blocking agents (2). It was therefore of interest to determine whether the lipolytic receptors of the rat resembled the receptors mediating glycogenolysis in the liver or those receptors mediating glycogenolysis in the heart and muscle of this species.

The intact rat has been extensively utilized for studies on the metabolic effects of sympathomimetic amines and their interaction with other endocrine hormones. Therefore, it appeared pertinent to clarify the receptors mediating lipolysis in the intact animal rather than utilize isolated adipose tissue as the experimental preparation. The receptors mediating epinephrine-induced increase in plasma free fatty acids were characterized first, on the basis of the relative potencies of epinephrine, norepinephrine, and isoproterenol, and second, on the ability of various adrenergic blocking agents (MJ-1999, Dibozane, Dibozane plus MJ-1999, dihydroergotamine, and *N*-isopropylmethoxamine) to antagonize the epinephrine-induced increase in plasma free fatty acids.

Methods. White male rats weighing 200-250 g, after a fast of approximately 24 hr, were anesthetized with a combination of pentobarbital sodium (15 mg/kg) and barbital sodium (200 mg/kg). All compounds were administered intraperitoneally.

At the specified times, the animals were sacrificed under anesthesia by decapitation. Blood was collected in heparinized, polyethylene beakers only during the first 10 sec immediately following decapitation.

The adrenergic blocking agents dihydroergotamine[3] (5 mg/ml in propylene glycol), Dibozane[3] [1,4-bis(1,4-benzodioxane-2-methyl) piperazine] (5 mg/ml in 0.4% phosphoric acid), and *N*-isopropylmethoxamine[3] (5 mg/

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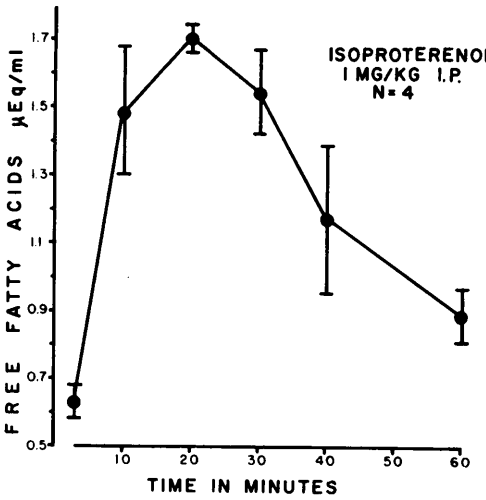

FIG. 1. Time–response data on isoproterenol-induced lipolysis: plasma free fatty acids concentrations are plotted on the ordinate. The blood was sampled 10, 20, 30, 40, or 60 min after the administration of isoproterenol, 1.0 mg/kg i.p., as indicated on the abscissa. Each point represents the mean plasma free fatty acid level of 4 animals and the vertical lines indicate the standard error of the mean (± SE).

ml in 0.5% lactic acid) were administered in a dose of 10 mg/kg and MJ-1999[3] [4-(2-isopropylamine-1-hydroxyethyl) methane sulfonanilide] (10 mg/ml in saline) was administered in a dose of 20 mg/kg. Thirty min after the adrenergic blocking agent epinephrine or isoproterenol was administered intraperitoneally. Stock solutions of *l*-epinephrine bitartate, *l*-norepinephrine bitartate, or *dl*-isoproterenol hydrochloride containing 1 mg/ml in 0.01 *N* HCl were prepared at the beginning of each experiment and appropriate dilutions in 0.01 *N* HCl were made immediately prior to injection. Control animals, animals receiving blocking agent but not a sympathomimetic amine, and animals receiving a sympathomimetic amine but not a blocking agent were given corresponding volumes of isotonic saline or of 0.01 *N* HCl in place of the test compound. All doses are expressed in terms of the salt.

Plasma free fatty acids were determined by the method of Dole and Meinertz (3) as modified by Trout *et al.* (4).

Statistical significances of the differences were determined by the Student's *t* test or the analysis of variance as described by Snedecor (5). Values above the 5% level of probability were considered not significant.

Results. Time–response study of the effect of isoproterenol on plasma free fatty acids in the rat. The observed effects of sympathomimetic amines on free fatty acid release are dependent on the dose, route of administration and time of sampling. In order to determine the optimal time of blood sampling, a time–response study was made of plasma free fatty acids in response to isoproterenol. The results are presented in Fig. 1.

After the intraperitoneal administration of isoproterenol, 1 mg/kg, the plasma free fatty acids increased to more than double the control values within the first 10 min. After 20 min, the fatty acids began to decrease, but they were still significantly above the control values throughout the 60-min sampling period.

This time–response curve of isoproterenol-induced lipolysis showed that the maximum increase in plasma free fatty acids could be obtained by sampling the blood at 10–20 min after the intraperitoneal injection of the sympathomimetic amine, with the apparent peak of the response at 20 min.

Table I summarizes the effects of various doses of epinephrine, norepinephrine, and isoproterenol on plasma free fatty acid levels in the intact rat. The blood was sampled 10 min after the injection of the sympathomimetic amines, a time at which lipolysis is exceeding the rate of reesterification.

The rise in plasma free fatty acids in response to norepinephrine, epinephrine, and isoproterenol shows a significant difference in potencies at most but not all dose levels, but these differences were small. Thus, determination of the relative potencies of epinephrine, norephinephrine, and isoproterenol on elevation of plasma free fatty acids in the rat did not show sufficiently clear differences to be sure which is the most potent, and therefore these data do not help to clarify the type of receptor in adipose tissue.

Effect of MJ-1999 on isoproterenol-induced lipolysis. In order to determine whether iso-

TABLE I. Effect of Epinephrine, Norepinephrine, and Isoproterenol on Plasma Free Fatty Acids in the Intact Rat.[a]

Dose of catecholamine (mg/kg)	Plasma free fatty acids (μeq/liter)		
	Epinephrine	Isoproterenol	Norepinephrine
0	563 ± 48[a] (5)	563 ± 48[a] (5)	563 ± 48[a] (5)
0.01	558 ± 38[c] (10)	1157 ± 85[b] (10)	705 ± 23[b,c] (5)
0.03	924 ±109 (5)	836 ± 42 (5)	1124 ± 26[c] (5)
0.1	972 ± 47[c] (5)	1247 ± 82[b] (5)	1605 ±101[b] (5)
0.3	923 ± 59[c] (10)	1476 ± 79[b] (5)	1794 ±187[b] (5)
1.0	1322 ± 72 (10)	1366 ± 72 (5)	1778 ± 45[b,c] (5)
3.0	1987 ± 45 (2)		

[a] Blood samples were obtained 10 min after the intraperitoneal injection of the sympathomimetic amines; mean ± SE; number of rats given in parentheses.

[b] Significantly different from epinephrine ($p < .05$).

[c] Significantly different from isoproterenol ($p < .05$).

proterenol-induced lipolysis was mediated by the β adrenergic receptor, the effect of the β adrenergic blocking agent, MJ-1999, in a dose of 20 mg/kg, was examined for its ability to antagonize isoproterenol effects. The results are summarized in Fig. 2.

MJ-1999, when given alone, produced no significant change in plasma free fatty acids. In keeping with the time–response study, 20 min after isoproterenol, 1 mg/kg, the free fatty acids were increased to 260% of the control value. In a dose of 0.5 mg/kg, isoproterenol increased the plasma free fatty acids to 170% of the control value.

MJ-1999 did not inhibit isoproterenol-induced lipolysis in the intact rat. In fact, MJ-1999 produced a significant potentiation of the rise in plasma free fatty acids produced by the smaller dose of isoproterenol. These results indicate β receptors do not mediate isoproterenol-induced lipolysis in the intact rat.

Effect of MJ-1999, Dibozane, and MJ-1999 plus Dibozane on free fatty acid release produced by epinephrine. In order to characterize further the receptor(s) mediating lipolysis, the effects of the β receptor blocking agent, MJ-1999, the α receptor blocking agent, Dibozane, and a combination of the α and β receptor blocking agents were studied on the free fatty acid release produced by epinephrine. The necessity for a combination of α plus β receptor blocking agents for inhibiting the response of the intestine to epinephrine was discovered by Ahlquist and Levy (6).

The results obtained in these experiments are presented in Fig. 3. Epinephrine, 0.5 mg/kg i.p., produced a marked rise in free fatty acids. No significant change in the plasma free fatty acid level occurred following the administration of MJ-1999, 20 mg/kg, Dibozane 10 mg/kg or a combination of MJ-1999 plus Dibozane. Neither MJ-1999, nor Dibozane nor the combination of Dibozane plus MJ-1999 diminished the effect of epinephrine on plasma free fatty acids. These results indicated that lipolysis in the rat is not mediated by α, by β, or by a combination of α plus β receptors.

Effects of dihydroergotamine and N-isopropylmethoxamine on epinephrine-induced lipolysis. Dihydroergotamine and *N*-isopropylmethoxamine, agents from two groups of compounds which were previously reported to be effective inhibitors of epinephrine-induced lipolysis in the rat (7–9), were tested against epinephrine-induced lipolysis. The results are presented graphically in Fig. 4.

Dihydroergotamine, when given alone, produced a small, but significant decrease in plasma free fatty acids; *N*-isopropylmethoxamine produced a small, insignificant decrease. Dihydroergotamine, 10 mg/kg i.p., produced a complete inhibition of the epine-

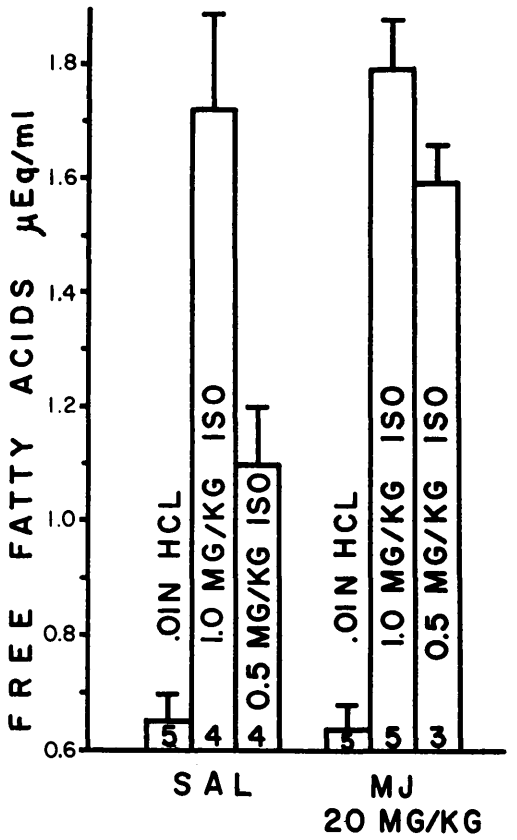

FIG. 2. Effect of MJ-1999 on isoproterenol-induced lipolysis: each bar represents the plasma free fatty acid content and the vertical lines indicate the standard error of the mean (± SE). The adrenergic blocking agent was administered 30 min prior to the intraperitoneal injection of isoproterenol. The animals were sacrificed 20 min after isoproterenol. The number of animals in each group is shown at the bottom of the column.

phrine-induced rise in plasma free fatty acids. *N*-Isopropylmethoxamine, 10 mg/kg i.p., had no significant effect on epinephrine-induced lipolysis.

To eliminate the possibility that propylene glycol (PG), the vehicle for dihydroergotamine, could be responsible for the inhibition of lipolysis, we studied the effect of propylene glycol on the rise in plasma free fatty acid produced by epinephrine. Figure 4 shows that propylene glycol produced no significant influence on the epinephrine-induced elevation of plasma free fatty acids.

Discussion. From the present studies in the intact rat, the lipolytic receptors appeared insensitive to the classical α and β adrenergic blocking agents. Whereas a dose of 1 mg/kg of MJ-1999 effectively inhibits epinephrine-induced lipolysis in the intact dog (10), in the present study 20 times this dose of MJ-1999 did not reduce the rise in plasma free fatty acids in the intact rat. A dose of Kö 592, fifty times that dose of MJ-1999 required for inhibition of lipolysis in the dog, did inhibit norepinephrine-induced lipolysis in the intact rat (11). This marked difference in sensitivity does not permit the classification of the

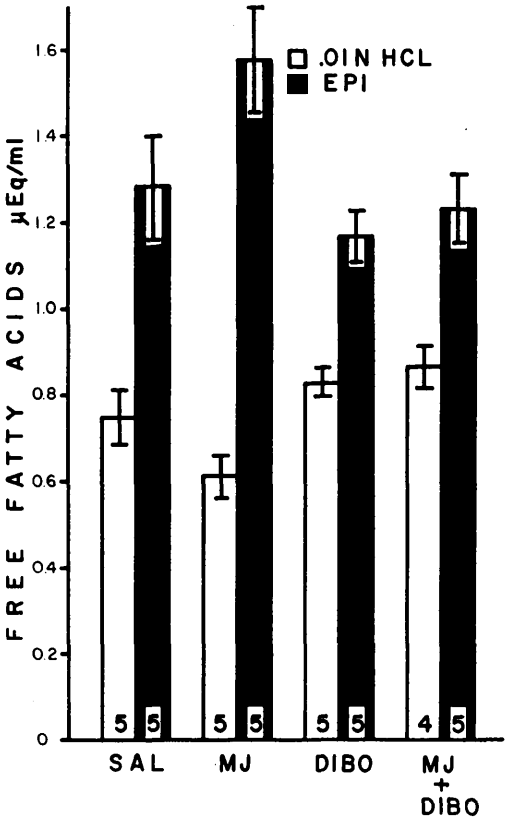

FIG. 3. Effect of MJ-1999, Dibozane, and MJ-1999 plus Dibozane on epinephrine-induced lipolysis: each bar represents the plasma free fatty acid content and the vertical lines indicate the standard error of the mean (± SE). MJ-1999, 20 mg/kg, Dibozane, 10 mg/kg, and a combination of the two agents were administered 30 min prior to the intraperitoneal injection of epinephrine, 0.5 mg/kg. The animals were sacrificed 20 min after epinephrine. The number of animals for each group is shown at the bottom of the column.

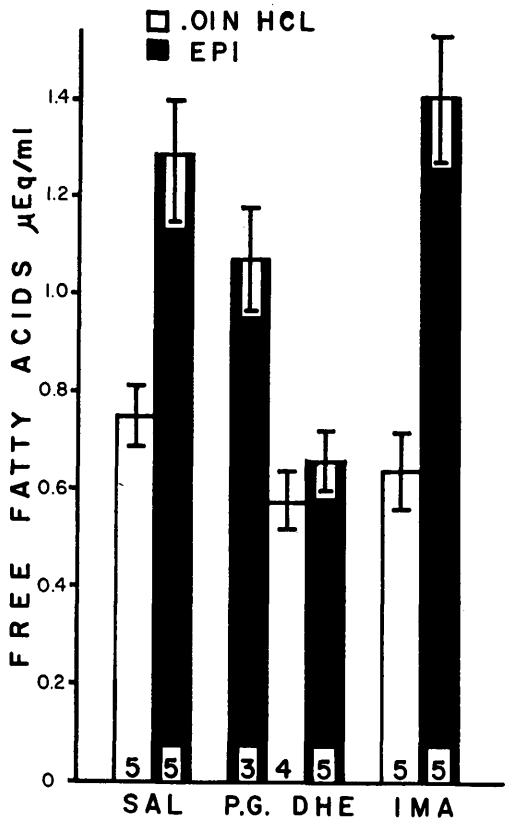

FIG. 4. Effect of dihydroergotamine and *N*-isopropylmethoxamine on epinephrine-induced lipolysis: each bar represents the plasma free fatty acid content and the verticle lines indicate the standard error of the mean (± SE). Dihydroergotamine, 10 mg/kg, *N*-isopropylmethoxamine, 10 mg/kg, or propylene glycol (PG) was administered 30 min prior to the intraperitoneal injection of epinephrine, 0.5 mg/kg. The animals were sacrificed 20 min after epinephrine. The number of animals for each group is shown at the bottom of each column.

rat lipolytic receptors as "typical" α or β receptors, nor can they be a combination of the two types, since neither the β antagonist MJ-1999, the α antagonist Dibozane, nor a combination of MJ-1999 plus Dibozane inhibited epinephrine-induced lipolysis. The characteristics of the receptors for epinephrine-induced free fatty acid release in the rat are similar to those of the receptors for epinephrine-induced liver glycogenolysis in this species (2). These receptors are characterized by their blockade by dihydroergotamine, but not by the more selective agents which block α and/or β adrenergic receptors.

The rat liver glycogenolytic receptors appear to differ from the lipolytic receptors in that they have a much higher affinity for epinephrine than for isoproterenol (2). In the present study and in studies on isolated epididymal fat pads (11–13), epinephrine and isoproterenol appeared about equally potent. In the dog, also, the relative potencies of epinephrine, norepinephrine, and isoproterenol are not consistent with the relative potencies characteristic of either α or β receptor activation (14, 15). However, it is now clear that β, rather than α, adrenergic blocking agents are the effective inhibitors of epinephrine-induced lipolysis in the dog (14–17), in man (18, 19), and in cat (20). It is noteworthy that catecholamine-induced hyperglycemia and lipolysis in the dog and cat are both activated through β receptors, whereas in man the β receptor controls the activation of only lipolysis. The hepatic glycogenolytic receptors of man appear similar to the receptors mediating epinephrine-induced lipolysis and glycogenolysis in the rat (2) in that they are blocked by neither α nor β adrenergic blocking agents (18), but dihydroergotamine is a fairly potent antagonist (21). Since dihydroergotamine is also effective in blocking the liver adrenergic receptor for glycogenolysis, the observation by Northrop and Parks (22) that DHE blocks the effect of cyclic AMP may explain this effect of DHE in adipose tissue.

The present *in vivo* studies on the receptors mediating lipolysis in the rat are not in complete agreement with studies utilizing isolated rat adipose tissue. In isolated tissue the lipolytic effect has been reported to be reduced by high concentrations of both types of blocking agents. However, it is apparent that β blocking agents are more effective antagonists of lipolysis in isolated tissue than are the α adrenergic blocking agents (10, 23–26). Generally, both mechanical and metabolic receptors in various organs and species differ in their relative potencies to sympathomimetic amines and to their relative susceptibility to blockade by a given blocking agent. Therefore, it may well be that the

adrenotropic receptor in rat adipose tissue more closely resembles a β receptor, but that it differs from the "typical" β receptor in its relative insensitivity to β adrenergic antagonists, both *in vitro* and *in vivo*. That rat adipose tissue is quite resistant to blockade by other agents which are effective antagonists of epinephrine-induced lipolysis in other species is evidenced by the high concentration of *N*-isopropylmethoxamine required to inhibit epinephrine-induced lipolysis in isolated rat adipose tissue (9), and in the present study, by the lack of inhibition of *N*-isopropylmethoxamine of the rise in plasma free fatty acids produced by epinephrine in the intact rat.

It is now apparent that the lipolytic effect of catecholamines is mediated at least in part through the adenyl cyclase system (23). However, the relationship of the adenyl cyclase system to the β and/or α adrenergic receptors is yet only speculative. The β adrenergic receptors appear closely related to the adenyl cyclase system. Less direct evidence is available to support the view that α adrenergic receptors are also related to adenyl cyclase (27). It is possible that the resistance of the rat lipolytic receptor to the "classical" adrenergic blocking agents results from a slight modification in the character of the receptor (26) or possibly in the adenyl cyclase system. However, the many other factors which regulate lipolysis and the flux of fatty acids across adipose cells cannot be eliminated. It is pertinent that the present studies do emphasize the resistance of the lipolytic receptors in the intact rat to "classical" adrenergic blocking agents, but their relative sensitivity to dihydroergotamine since both adrenergic agonists and antagonists are frequently utilized in this species for studies on hormonal control of metabolism.

Summary. White male rats, fasted approximately 24 hr, were anesthetized with a mixture of pentobarbital sodium and barbital sodium. All drugs were given intraperitoneally. Blood samples were obtained by decapitating the anesthetized animals and free fatty acids were determined on the plasma. Ten min after the administration of the sympathomimetic amines, there was no marked differences in the relative potencies of *l*-epinephrine, *l*-norepinephrine, and *dl*-isoproterenol. The adrenergic blocking agent, MJ-1999, did not inhibit the rise in plasma free fatty acids produced by isoproterenol. Neither MJ-1999, Dibozane, nor MJ-1999 plus Dibozane inhibited the rise in plasma free fatty acids produced by epinephrine. Dihydroergotamine completely inhibited epinephrine-induced lipolysis whereas *N*-isopropylmethoxamine had no effect. From these results, it is concluded that epinephrine-induced lipolysis is relatively resistant to blockade by the "classical" α and β adrenergic blocking agents or a combination of an α and a β adrenergic blocking agent, but lipolysis is effectively inhibited in the intact rat by dihydroergotamine.

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A Study of Ganglionic Denervation Supersensitivity Using McN-A-343 and Histamine as Ganglion Stimulating Agents* (33760)

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An increase in sensitivity of the superior cervical ganglion cells to acetylcholine has been purported to occur several days after cutting the preganglionic cervical sympathetic trunk (1–4). More recent evidence from electrophysiological experiments, however, strongly contradicts these earlier studies (5, 6).

The earlier experiments using the contraction of the nictitating membrane as a measure of ganglionic stimulation are difficult to interpret, because acetylcholine may also stimulate the nictitating membrane directly (7). To avoid this problem, two ganglion stimulating agents, which do not directly contract the nictitating membrane, viz, histamine (8, 9) and McN-A-343 (10), [4-(3-chlorphenylcarbamoyloxy)-2-butynyl trimethylammonium chloride], were used in the present study to investigate the possibility that the superior cervical ganglion is an exception to the classical concept of denervation supersensitivity (2).

Methods. Adult cats of either sex were anesthetized with sodium pentobarbital, 40 mg/kg, administered intraperitoneally. Chronic denervation of either the left or right superior cervical ganglion was performed 2–3 weeks prior to the actual experiment. An incision was made parallel to the trachea, the carotid sheath was isolated, and 1-cm segment of the cervical sympathetic trunk, located approximately 3 cm proximal to the ganglion, was removed. The incision was then closed with wound clips. The surgical procedure was followed by an intramuscular injection of 150,000 units of benzathine penicillin (Bicillin).

On the day of the experiment a tracheal cannula was inserted and the intact (control) ganglion on the contralateral side was acutely denervated. The animal was then bilaterally adrenalectomized to prevent the release of catecholamines by histamine or McN-A-343. Both nictitating membranes were attached to separate force-displacement transducers

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