Inhibition of the lipolytic action of β-adrenergic agonists in human adipocytes by α-adrenergic agonists

Elizabeth E. Wright and Evan R. Simpson

Cecil H. and Ida Green Center for Reproductive Biology Sciences and the Departments of Obstetrics and Gynecology and Biochemistry, University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, TX 75235

Abstract The aim of this study was to define the role of the α-adrenergic receptor in the regulation of lipolysis by human adipocytes. Glycerol production by isolated human adipocytes was stimulated by the pure β-adrenergic agonist isoproterenol in a dose-dependent fashion. This stimulation of lipolysis was inhibited by the α-adrenergic agonists methoxamine, phenylephrine, and clonidine. Epinephrine-stimulated lipolysis was potentiated by the α-adrenergic antagonists, dihydroergocryptine, phentolamine, phenoxybenzamine, and yohimbine. Whereas the attenuation of β-adrenergic agonist-stimulated lipolysis by α-adrenergic agonists was reversed completely by the α_2-adrenergic antagonist yohimbine, the α_1-antagonist prazosin did not reverse such attenuation. It is concluded that α-adrenergic agonists act as antilipolytic agents in human adipocytes and that this action may result from the interaction of these compounds with a population of α_2-adrenergic receptors. —**Wright, E. E., and E. R. Simpson.** Inhibition of the lipolytic action of β-adrenergic agonists in human adipocytes by α-adrenergic agonists. *J. Lipid Res.* 1981. **22:** 1265–1270.

Supplementary key words lipolysis · adrenergic receptors · α-adrenergic antagonists

The presence of α-adrenergic receptors in human subcutaneous tissue was suggested first by Burns and Langley (1) and Burns, Langley, and Robison (2) who found that the rate of lipolysis stimulated by epinephrine in human adipocytes was much less than the rate of isoproterenol-stimulated lipolysis. In this regard, it is important to note that human adipocytes contain a large population of α-adrenergic receptors as well as β-adrenergic receptors (3–9). Previously we found that the binding capacity of human adipocytes for α-adrenergic ligands was approximately seven times that of the binding capacity of these cells for β-adrenergic ligands (10). The presence of a large population of α_2-adrenergic receptors in human fat cells was confirmed by studies of the binding of [^{3}H]dihydroergocryptine (DHEC) and [^{3}H]p-aminoclonidine (PAC) to preparations of plasma membranes of human adipocytes.

The presence of a high concentration of α_2-adrenergic receptors on the surface of human adipocytes may provide an explanation for the relatively small stimulation of lipolysis that is elicited by epinephrine compared to that which is elicited by isoproterenol. It has been suggested that the α-adrenergic receptor may mediate an antilipolytic action (1, 2, 11–15) and it has been observed that the α-adrenergic agonists, methoxamine and phenylephrine, attenuate the stimulation of lipolysis elicited by β-agonists in hamster adipocytes (14–16). Since epinephrine is a mixed α- and β-adrenergic agonist, the net lipolytic action of epinephrine could be expected, therefore, to be less than that of isoproterenol, a pure β-agonist. Indeed, phentolamine, an α-adrenergicant agonist, potentiated the lipolytic action of epinephrine in human adipocytes (1). Similar results were obtained using hamster adipocytes and norepinephrine (11, 12).

To investigate further the role of α-adrenergic receptor-mediated phenomena in the regulation of lipid metabolism in human adipocytes, the present study was conducted to evaluate the effects of the α-adrenergic agonists, clonidine, methoxamine, and phenylephrine, and those of the α-adrenergic antagonists, phentolamine, yohimbine, prazosin, and phenoxybenzamine on lipolysis in these cells. Portions of this work have appeared in abstract form (17).

METHODS

Human adipose tissue was obtained from the anterior abdominal wall of women undergoing elective laparotomy. Adipocytes were dispersed by the method

Abbreviations: DHEC, dihydroergocryptine; PAC, p-aminoclonidine.

of Rodbell (18). The tissue was finely minced at room temperature and then incubated in 2 ml of Krebs-phosphate buffer, pH 7.4, containing bovine serum albumin ((4% w/v), BSA; Miles Pentex, fraction V), and 1 mg of collagenase (CLS grade, Millipore Corporation, Freehold, NJ) per gram wet weight of tissue for 1 hr at 37°C in a shaking incubator. The cell suspension was filtered through fine mesh nylon. The adipocytes were washed three times in Krebs-phosphate-BSA buffer. The number of cells in an aliquot was quantified using a hemocytometer. The cells were resuspended in Krebs-phosphate-BSA buffer to a final concentration of 1×10^6 adipocytes per ml of buffer. Aliquots (1 ml) of the suspension of adipocytes were placed into plastic tubes and the various agonists and antagonists were added to the incubation buffer to achieve concentrations as indicated in the text. The adipocytes were incubated at 37°C for 1 hr in a shaking incubator in an atmosphere of O_2 (95%), CO_2 (5%). Following incubation, an aliquot (0.5 ml) of buffer infranatant fluid was removed from each incubation mixture for measurement of the amount of glycerol released from the cells. Glycerol was measured by monitoring NADH generation according to a modified version of the enzymatic method of Wieland (19). The assay was conducted in a final volume of 3.0 ml per assay tube. All measurements were performed in duplicate and each experiment was conducted at least twice with similar results.

Epinephrine bitartrate, L-isoproterenol hydrochloride, L-phenylephrine hydrochloride, and yohimbine hydrochloride were obtained from Sigma Chemical Company, St. Louis, MO. Phentolamine mesylate was obtained from CIBA, Summit, NJ. L-Propranolol hydrochloride was obtained from Ayerst Laboratories Inc., New York, NY. Clonidine hydrochloride, methoxamine hydrochloride, prazosin hydrochloride and phenoxybenzamine were gifts from Dr. Ladislav Krulich, Department of Physiology, University of Texas Health Science Center at Dallas. Dihydroergocryptine was obtained from Sandoz Pharmaceuticals, East Hanover, NJ. Glycerol kinase, glycerol-3-phosphate dehydrogenase, NAD free acid (Grade 1, 100%), and ATP, crystalline disodium salt, were obtained from Boehringer Mannheim, Indianapolis, IN. Stock solutions were prepared immediately before use and all compounds were dissolved in water.

RESULTS

Previously, it was shown by other investigators (1) that lipolysis stimulated by epinephrine in human adipocytes can be potentiated by the α-adrenergic antagonist phentolamine. These results are indicative

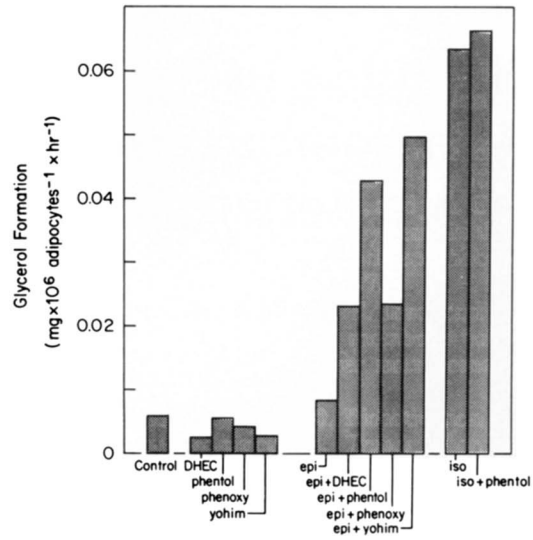

Fig. 1. Effect of α-adrenergic antagonists on epinephrine- and isoproterenol-stimulated lipolysis. Glycerol formation is expressed as mg × 10^6 adipocytes^{-1} × hr^{-1}. Values shown are the average of duplicate determinations. The control (basal) value is that found for lipolysis in untreated cells. Concentrations of the agents used were: epinephrine (epi), 1×10^{-6} M; isoproterenol (iso), 1×10^{-6} M; DHEC, 8.1×10^{-5} M; phentolamine (phentol), 8.1×10^{-5} M; phenoxybenzamine (phenoxy), 8.1×10^{-5} M; yohimbine (yohim), 8.1×10^{-5} M.

that the α-adrenergic receptors present in human adipocytes may modulate an antilipolytic action. The potentiation of epinephrine-stimulated lipolysis by α-adrenergic antagonists was examined more thoroughly and the results of these studies are presented in **Fig. 1.** The α-adrenergic antagonists DHEC, phentolamine, phenoxybenzamine, and yohimbine, each at a concentration at 8.1×10^{-5} M, enhanced the lipolytic response of human adipocytes to epinephrine (1×10^{-6} M). Whereas all of the α-adrenergic antagonists potentiated the lipolytic stimulation by epinephrine, the α_2-adrenergic antagonist yohimbine and mixed antagonist phentolamine were more effective potentiating agents than were the mixed antagonist DHEC or the α_1-antagonist phenoxybenzamine. Phentolamine had little or no effect on lipolysis stimulated by isoproterenol (1×10^{-6} M), a pure β-agonist. None of the antagonists affected basal rates of lipolysis.

The finding of a large population of α-adrenergic receptors in human adipocytes (10) was suggestive of the possibility that α-adrenergic agonists such as clonidine, methoxamine, or phenylephrine would inhibit the stimulation of lipolysis brought about by pure β-adrenergic agonists. The stimulation of lipolysis induced by isoproterenol in various concentrations in the presence or absence of clonidine (8.1×10^{-5} M) is shown in **Fig. 2A.** In the absence of clonidine, the

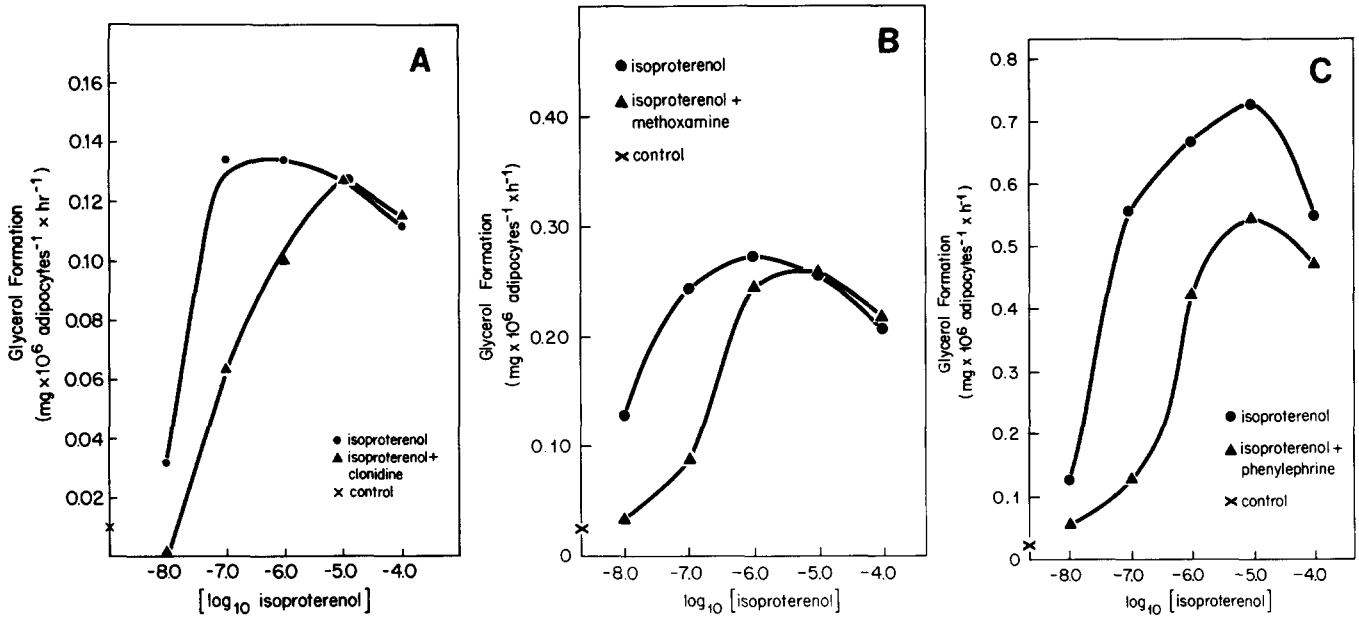

Fig. 2. A. Effect of clonidine on isoproterenol-stimulated lipolysis. B. Effect of methoxamine on isoproterenol-stimulated lipolysis. C. Effect of phenylephrine on isoproterenol-stimulated lipolysis by human adipocytes. Glycerol formation is expressed as mg × 10^6 adipocytes^{-1} × hr^{-1}. Each point is the average value of duplicate determinations. The control (basal) value is that found for lipolysis in untreated cells. The concentration of all agonists was 8.1 × 10^{-5} M.

half-maximum stimulation of lipolysis brought about by isoproterenol was achieved at a concentration of isoproterenol of approximately 2.5 × 10^{-8} M. In the presence of clonidine, the concentration of isoproterenol required to achieve half-maximal stimulation of lipolysis increased to 4.7 × 10^{-7} M. Inhibition of isoproterenol-stimulated lipolysis by clonidine was overcome as the concentration of isoproterenol was increased. In both the presence and absence of clonidine, isoproterenol in high concentrations inhibited lipolysis, presumably because isoproterenol binds with low affinity to α-adrenergic receptors (10) which may mediate an antilipolytic effect. In similar experiments using methoxamine (8.1 × 10^{-5} M, Fig. 2B) and phenylephrine (8.1 × 10^{-5} M, Fig. 2C) as α-adrenergic agonists, isoproterenol-stimulated lipolysis was attenuated. The concentration of isoproterenol that was required to achieve half-maximum stimulation of lipolysis, in the absence of methoxamine and phenylephrine, was 5.0 × 10^{-8} M, whereas in the presence of methoxamine and phenylephrine it was 3.2 × 10^{-7} M and 7.5 × 10^{-7} M, respectively. As in the case of clonidine, in both the presence and absence of the α-agonists methoxamine and phenylephrine, high concentrations of isoproterenol were inhibitory of lipolysis.

The effects of these α-adrenergic agonists, in various concentrations, on lipolysis stimulated by isoproterenol (1 × 10^{-7} M) are depicted in **Fig. 3.** In each case, lipolysis was inhibited as the concentration of the α-adrenergic agonist was increased. Clonidine and methoxamine were more effective inhibitory agents than phenylephrine. Inhibition of lipolysis by clonidine and methoxamine was first detected with agonist concentrations of 2.2 × 10^{-6} M and 4.6 × 10^{-6} M, respectively. Inhibition by phenylephrine was first detectable at a ten-fold higher concentration of agonist, namely 2.2 × 10^{-5} M.

It is apparent that α-adrenergic antagonists cause a marked enhancement of epinephrine-stimulated lipolysis in human adipocytes. This finding can be interpreted to mean that these antagonists block the α-adrenergic action of epinephrine and that through such a process the inhibitory component of epinephrine action on lipolysis can be overcome. Consequently, we elected to ascertain whether α-adrenergic antagonists such as yohimbine and prazosin would block the inhibitory action of α-adrenergic agonists on isoproterenol-stimulated lipolysis. The adipocytes were incubated with isoproterenol (1 × 10^{-6} M), and clonidine in various concentrations in the absence and presence of yohimbine (8.1 × 10^{-5} M), an α_2-adrenergic antagonist (**Fig. 4A**). Yohimbine effectively reversed the inhibitory effect of clonidine on lipolysis stimulated by isoproterenol. When prazosin (8.1 × 10^{-6} M), an α_1-adrenergic antagonist, was substituted for yohimbine (Fig. 4B), there was no block of the inhibitory action of clonidine, although yohimbine at the same concentration was effective in reversing the action of clonidine.

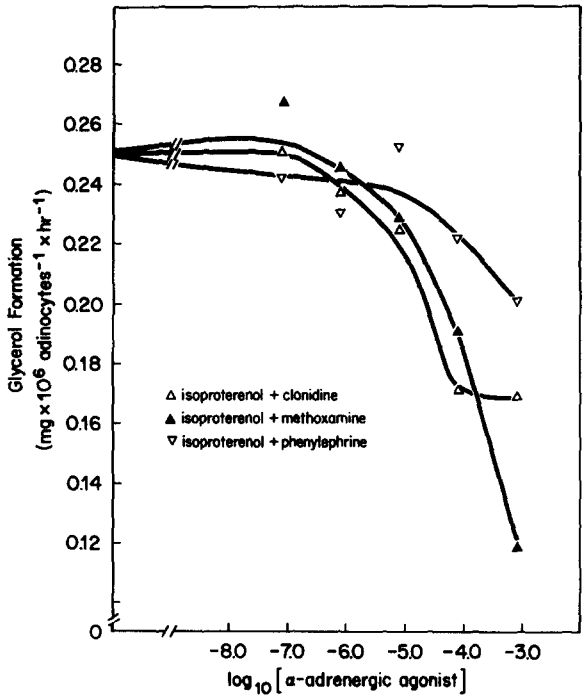

Fig. 3. Effect of clonidine, methoxamine and phenylephrine on isoproterenol-stimulated lipolysis. Glycerol formation is expressed as mg × 10^6 adipocytes^{-1} × hr^{-1}. Each point is the average of duplicate determinations. The concentration of isoproterenol was 1×10^{-7} M.

DISCUSSION

The results of this study are indicative that the α_2-adrenergic agonist clonidine, and the α_1-adrenergic agonists methoxamine and phenylephrine, attenuate the stimulation of lipolysis elicited by isoproterenol, a β-adrenergic agonist, in human adipocytes (Figs. 2A, 2B, 2C, 3, 4A, and 4B). It was observed (Figs. 2 and 3) that clonidine and methoxamine were slightly more effective inhibitory agents than phenylephrine. The inhibition of lipolysis by clonidine and methoxamine was overcome by isoproterenol in high concentrations. Moreover, isoproterenol alone, at high concentrations, also may have acted to inhibit lipolysis by virtue of its weak interaction with α-adrenergic receptors (10).

As was shown by Burns and Langley (1) and again in the present study (Fig. 1), α-adrenergic antagonists potentiate the lipolytic action of epinephrine on human adipocytes. It would seem a logical supposition that α-adrenergic antagonists would reverse the α-adrenergic agonist-induced inhibition of lipolysis stimulated by isoproterenol. Indeed, yohimbine, an α_2-adrenergic antagonist was effective in the complete reversal of the inhibitory effect of clonidine (Fig. 4A) whereas prazosin, an α_1-adrenergic antagonist, did not

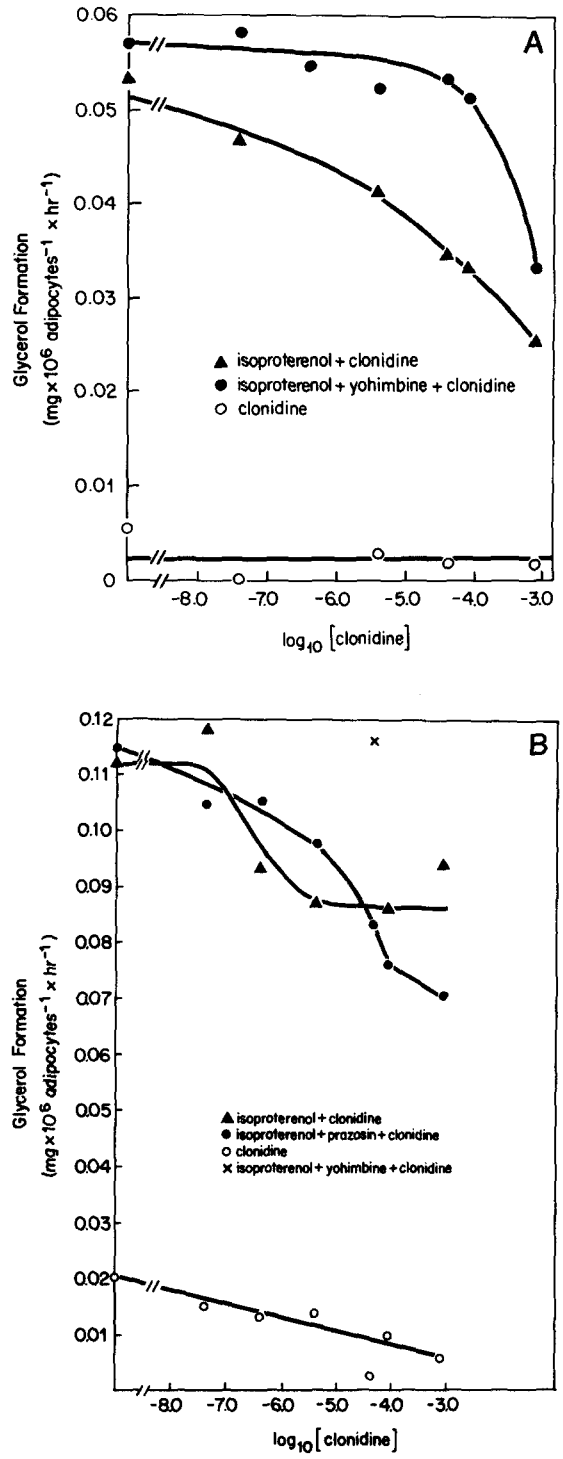

Fig. 4. A. Effect of the α_2-adrenergic antagonist yohimbine on clonidine-induced inhibition of isoproterenol-stimulated lipolysis. B. Effect of the α_1-adrenergic antagonist prazosin on clonidine-induced inhibition of isoproterenol-stimulated lipolysis. Glycerol formation is expressed as mg × 10^6 adipocytes^{-1} × hr^{-1}. Each point is the average of duplicate determinations. The concentrations of the agents used were: panel A: isoproterenol, 1×10^{-6} M; yohimbine, 8.1×10^{-5} M; panel B: isoproterenol, 1×10^{-6} M; yohimbine, 8.1×10^{-6} M; prazosin, 8.1×10^{-6} M.

block the inhibitory action of clonidine (Fig. 4B). In experiments utilizing hamster adipocytes, it was found that reduction of intracellular cyclic AMP concentrations by α-adrenergic agonists could be overcome by α-adrenergic antagonists (15, 16, 20).

The effect of α-adrenergic agonists on lipolysis by human adipocytes is strongly supportive of the conclusion that α_2-adrenergic receptors mediate an antilipolytic action (1, 2). Similar observations have been made in studies of hamster adipocytes (11–15) that have been shown to possess a mixed population of α_1- and α_2-adrenergic receptors (21, 22). The mechanism through which the α_2-adrenergic agonists cause inhibition of lipolysis remains unclear. There is evidence that is strongly indicative that binding of agonists to α_2-adrenergic receptors causes a decrease in cyclic AMP synthesis through inhibition of adenylate cyclase (23, 24). On the other hand, it is believed that binding to α_1-adrenergic receptors results in an increase in phosphatidylinositol turnover and calcium gating or mobilization (25–27). It is unclear how phenylephrine and methoxamine, α_1-adrenergic agonists, mediate their antilipolytic effects in human adipocytes. It is possible that the binding of methoxamine and phenylephrine to α_2-adrenergic receptors (10) will initiate inhibition of adenylate cyclase, or that their binding to the small population of α_1-adrenergic receptors on human adipocytes also initiates an antipolytic response. It has been demonstrated that GTP will lower the binding affinity of agonists to α-adrenergic receptors without affecting the affinity of α_1-adrenergic receptors (23, 28, 29). It is highly possible that the level of GTP in the adipocyte is sufficiently high that the affinity of α_2-adrenergic receptors for α_2-adrenergic agonists is lowered to the extent that both α_2- and α_1-adrenergic agonists bind to α_2-receptors with approximately equal affinity, and thus both are approximately equally as potent as antilipolytic agents. Further work is necessary to clarify this point. We have evidence that both clonidine and methoxamine will inhibit the activity of adenylate cyclase that has been stimulated by isoproterenol in the presence of GTP (data not shown). Schimmel, Serio, and McMahon (14) presented results that are indicative that α_1- as well as α_2-adrenergic agonists will lower cyclic AMP levels and inhibit lipolysis in hamster adipocytes.

The physiological role of α-adrenergic receptors in human adipocytes is a matter of speculation. It is possible that the ratio of functional α- and β-adrenergic receptors can be influenced by such factors as age and nutritional and hormone status. Lafontan (30) presented evidence that the α-adrenergic receptor population in adipose tissue of rabbits increases with age. Giudicelli, LaCasa, and Agli (31) found that there is a reduction in the number of α-adrenergic receptors in adipocytes of hyperthyroid hamsters. Finally, it appears that the number of α-adrenergic receptors in rabbit uterus and platelets decreases with estrogen treatment (32–34). These observations are suggestive that the number of functional α-adrenergic receptors in adipose tissue, as well as other tissues, may play a role in the changes in body weight and character that are associated with aging and hormonal dysfunction.

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