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Evidence for *Alpha*-Adrenergic Activation and Inactivation of Phosphorylase in Hamster Adipocytes

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SUMMARY

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Exposure of hamster adipocytes to the selective *beta*-adrenergic agonist isoproterenol or to the selective *alpha*-adrenergic agonist methoxamine increased the percentage of phosphorylase *a* activity. When present together, the responses to methoxamine and isoproterenol were nearly additive. Of the *alpha*-adrenergic antagonists studied, prazosin was the most effective at blocking methoxamine activation of phosphorylase, followed by yohimbine and phenoxybenzamine. In contrast to the stimulatory action of methoxamine, clonidine ($alpha_2$-specific) did not activate phosphorylase. Incubation of cells with 3-isobutyl-1-methyl xanthine (IBMX) increased phosphorylase *a* activity to levels observed with isoproterenol. When cells were exposed to methoxamine or clonidine alone with IBMX, the stimulatory action of IBMX on phosphorylase *a* activity was partially prevented. The inhibitory effect of methoxamine was increased by prazosin and prevented by yohimbine. These results show, therefore, that *alpha*-receptor activation can both increase and decrease phosphorylase *a* activity. These results serve to strengthen the view that *alpha*-adrenergic inhibition of phosphorylase is mediated by $alpha_2$-receptors whereas activation of phosphorylase is mediated by $alpha_1$-receptors and that *alpha*-receptor can both increase and decrease phosphorylase *a* activity. We suggest that these oppositely directed responses result from two different subtypes of *alpha*-adrenergic receptors present upon hamster adipocytes.

INTRODUCTION

In isolated adipocytes prepared from rats, Lawrence and Larner (1, 2) demonstrated the presence of both *alpha*- and *beta*-adrenergic components to catecholamine activation of phosphorylase and inactivation of glycogen synthase. The *beta*-adrenergic component is most likely mediated by increased accumulation of cyclic AMP with resultant increased cyclic AMP-dependent protein kinase activity (1). The *alpha*-adrenergic mechanism, however, is not associated with changes in cyclic AMP and may be mediated by changes in cytoplasmic calcium levels (2). In their studies, Lawrence and Larner (1, 2) did not determine whether *alpha*-adrenergic activation of phosphorylase and inactivation of glycogen synthase were mediated by $alpha_1$- or $alpha_2$-receptors. However, the two *alpha*-adrenergic agonists used by Lawrence and Larner, methoxamine and phenylephrine, have been shown to be preferential for $alpha_1$-receptors (3–5). García-Sáinz and Fain (6) subsequently reported blockade of methoxamine inactivation of glycogen synthase by prazosin, an $alpha_1$-site-preferential antagonist (7), and suggested mediation of this response by $alpha_1$-receptors. These responses in rat adipocytes are 10- to 100-fold less sensitive to methoxamine and phenylephrine than are responses to $alpha_1$-selective agonists in other more well-defined systems (4, 5, 8). For instance, *alpha*-adrenergic activation of phosphorylase in rat hepatocytes is detected at a phenylephrine concentration of 0.10 μM (8). In contrast, phenylephrine concentrations greater than 20 μM were necessary to activate phosphorylase in rat adipocytes (1).

Unlike studies on rat adipocytes, studies on hamster adipocytes have shown *alpha*-adrenergic responses that are antagonistic to *beta*-adrenergic responses. For example, exposure of hamster adipocytes to selective *alpha*-adrenergic stimuli causes inhibition of lipolysis (9), inhibition of protein kinase activity (10), and inhibition of cyclic AMP accumulation (11). Each of these actions is most likely a result of a primary inhibition of adenylate cyclase activity (12). The receptor mediating these re-

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sponses in hamster adipocytes has been shown by several laboratories to be more similar to *alpha*$_2$- than to *alpha*$_1$-receptors (11–13).

Alpha-adrenergic regulation of glycogen phosphorylase has not been studied in hamster adipocytes. Since other *alpha*-adrenergic responses of these cells are associated with a reduction in stimulated cyclic AMP formation, it would seem likely that *alpha*-adrenergic agonists would antagonize phosphorylase activation in hamster adipocytes. To test this hypothesis, the effects of the selective *alpha*-adrenergic agonists clonidine and methoxamine on basal and stimulated phosphorylase activities were measured in isolated hamster adipocytes.

METHODS

Materials. Collagenase (Type I) was obtained from Worthington Biochemical Corporation (Freehold, N. J.); isoproterenol, glycogen, glucose-1-phosphate, norepinephrine, AMP, bovine serum albumin (Type V), and Dowex 1 × 8 100–200 dry mesh in the chloride form were obtained from Sigma Chemical Company (St. Louis, Mo.). IBMX[1] was obtained from Aldrich Chemical Company (Milwaukee, Wisc.). Clonidine was a generous gift from Boehringer Ingelheim (Ridgefield, Conn.). Insulin was obtained from Eli Lilly Inc. (Indianapolis, Ind.). Propranolol was obtained from Ayerst Laboratories (New York, N. Y.); phenoxybenzamine was obtained from Smith Kline & French (Philadelphia, Pa.). Yohimbine was obtained from ICN Pharmaceuticals Inc. (Plainview, N. Y.), and methoxamine was obtained from Burroughs-Wellcome (Research Triangle Park, N. C.). [*U*-^{14}C]Glucose-1-phosphate was obtained from Amersham Corporation (Arlington Heights, Ill.). All other chemicals were of reagent grade. The glycogen was purified by passing a 10% solution through a mixed-bed ion exchange resin (Amberlite MB-3 from Bio-Rad Laboratories, Richmond, Calif.), precipitated in 66% ethanol and recovered by centrifugation.

Preparation and incubation of fat cells. Male golden hamsters weighing between 90 and 100 g (Charles River Breeding Laboratories, Lakeview, N. J.) were fed Purina laboratory chow and had access to tap water ad libitum for at least 1 week before they were used. The animals were killed by cervical dislocation, and epididymal fat bodies were removed. Adipocytes were prepared by collagenase digestion in Krebs-Ringer phosphate buffer, pH 7.4, containing bovine serum albumin, 30 mg/ml, as described previously (9, 11). The fat cells were suspended in 10 ml of buffer of identical composition per gram of original tissue and incubated with shaking at 37° under an air atmosphere. Unless otherwise stated, each incubation lasted for 20 min; hormones and other agents were present for the last 4 min. Generally between 75 and 100 mg of cells were present in each incubation vessel.

Assay of phosphorylase. Following incubation, the cells were separated from the medium by flotation in silicone [Prosil-28, PCR Research Chemicals, Inc. (Gainesville, Fla.)]-coated Pasteur pipettes and homogenized in 0.3 ml of 100 mM KF and 10 mM EDTA (pH 7.0) at 4° using a Potter-Elvehjem tissue grinder fitted with a Teflon pestle. The homogenates were centrifuged at 12,500 × *g* for 15 min at 4° and the lipid-free infranatant was collected by aspiration and assayed for glycogen phosphorylase in the direction of glycogen synthesis from [^{14}C]glucose-1-phosphate as described by Gilboe *et al.* (14), using a 180-min incubation period. Radioactive glycogen formed during the assay was separated from glucose-1-phosphate by passing the reaction mixture through a column (1.2 × 25 cm) of Dowex 1 × 8 in the hydroxyl form eluted with two successive 0.5-ml washes of water (15). Preliminary studies showed that phosphorylase activity was linear with time for up to 4 hr. Phosphorylase activity is expressed as %*a* phosphorylase relative to the activity measured in the presence of 2 mM AMP.

RESULTS

The effect of glucose, insulin, norepinephrine, isoproterenol, ACTH, IBMX, clonidine, or methoxamine on phosphorylase activity is presented in Table 1. Basal phosphorylase activity in cells incubated for 20 min in the absence of hormones and glucose was approximately 43%*a*. Over the course of these studies, basal activity was occasionally as low as 29.5%*a* and as high as 63.9%*a*. As has been reported by others using rat adipocytes (1, 16–18), exposure of hamster adipocytes to isoproterenol, norepinephrine, ACTH, or IBMX produced a large increase in the percentage of phosphorylase *a* activity. When glucose was added, with or without insulin, the activity of phosphorylase *a* was reduced.

TABLE 1

Effect of hormone on phosphorylase activity in hamster adipocytes

Hamster adipocytes were incubated at 37° for 16 min. Various hormonal agents were then added and the incubation was continued for 4 min. The incubation was then terminated and the activities of phosphorylase were assessed as described under Methods. Each value is the mean ± standard error of the mean of *n* experiments, each performed on a different day. The difference between paired values ± standard error of the mean is represented by Δ.

Addition	Control	Phosphorylase, %*a* activity (−/+ 2 mM AMP)		
		+Hormone	Δ	n
Isoproterenol, 1 μM	41.3 ± 1.5	59.3 ± 1.6	18.0 ± 1.4[a]	25
ACTH, 30 mU/ml	54.3 ± 6.3	72.7 ± 2.0	18.4 ± 4.3[b]	3
Norepinephrine, 1 μM	43.1 ± 1.3	69.7 ± 2.8	26.6 ± 2.9[a]	6
IBMX, 0.1 mM	42.2 ± 2.2	64.4 ± 2.6	22.2 ± 1.8[a]	13
Glucose, 5 mM	40.2 ± 2.0	31.9 ± 2.2	−8.3 ± 2.3[a]	14
Insulin, 100 μU/ml	42.3 ± 2.0	43.6 ± 1.7	1.3 ± 1.5	12
Glucose, 5 mM + insulin, 100 μU/ml	43.3 ± 2.5	29.1 ± 2.6	−14.2 ± 3.3[a]	11
Methoxamine, 1 μM	46.6 ± 6.2	45.0 ± 3.9	−1.6 ± 2.8	4
Methoxamine, 10 μM	43.5 ± 1.2	46.4 ± 1.3	2.9 ± 1.1[c]	37
Methoxamine, 100 μM	48.6 ± 1.4	53.9 ± 1.3	5.4 ± 1.6[a]	41
Clonidine, 10 μM	48.0 ± 2.3	48.8 ± 2.3	0.8 ± 2.1	11
Clonidine, 100 μM	42.4 ± 2.7	44.0 ± 1.8	1.6 ± 2.3	4

[a] Significantly different from zero, $p < 0.01$.
[b] Significantly different from zero, $p < 0.05$.
[c] Significantly different from zero, $p < 0.02$.

[1] The abbreviations used are: IBMX, 3-isobutyl-1-methyl xanthine; %*a*, percentage of phosphorylase activity measured in the absence of added AMP.

Lawrence and Larner (1) reported activation of phosphorylase in rat adipocytes with the selective *alpha*-adrenergic agent methoxamine. In hamster adipocytes, methoxamine similarly increased the percentage of phosphorylase *a* activity. The minimum effective concentration of methoxamine was found to be 10 μM. However, the activation of phosphorylase caused by 10 μM methoxamine was much smaller than the activation produced by one-tenth as much isoproterenol or norepinephrine. Although the small response to methoxamine was statistically significant at the $p < 0.05$ level, this became apparent only after a relatively large number of experiments was analyzed. Increasing the concentration of methoxamine to 100 μM produced a slightly greater activation of phosphorylase, but the response is still much less than that produced by 1.0 μM isoproterenol or norepinephrine. In contrast to methoxamine, clonidine did not affect the activation state of phosphorylase, even when it was present at 100 μM.

The increase in phosphorylase *a* activity caused by methoxamine was not evident in every experiment. An increase in phosphorylase *a* activity in response to 10 μM methoxamine was detected in 26 of 37 experiments and in 36 of 41 experiments when 100 μM methoxamine was used. In contrast, the activation of phosphorylase in response to isoproterenol, ACTH, norepinephrine, or IBMX was evident in every experiment in which these agents were present. We investigated the possibility that longer or shorter periods of incubation with methoxamine may expose a greater activation of phosphorylase. However, when the incubation was varied from 1 to 10 min, no greater or more consistent response to methoxamine was detected.

Table 2 summarizes the results obtained from experiments in which adipocytes were exposed to methoxamine or clonidine singly or together with 1.0 μM isoproterenol. By itself, methoxamine produced a small increase in the activity of phosphorylase *a*. When present along with isoproterenol, methoxamine caused the activity of phosphorylase *a* to increase over that seen in the presence of isoproterenol alone. Clonidine, on the other hand, had no effect on phosphorylase *a* activity in the absence of isoproterenol and did not alter the activation of phosphorylase caused by isoproterenol. Since the response to methoxamine was quite small we considered the possibility that the natural agonist norepinephrine might elicit a larger *alpha*-adrenergic response. To investigate this possibility, the effect of norepinephrine on phosphorylase *a* activity was assessed in the presence of propranolol, which prevented phosphorylase activation consequent to *beta*-adrenergic receptor activation. Norepinephrine activation of phosphorylase is not fully blocked by propranolol. A small increase in phosphorylase *a* activity, approximating that seen in response to methoxamine, persisted in the presence of 100 μM propranolol (data not presented).

TABLE 2

Effect of methoxamine and clonidine on phosphorylase activity in hamster adipocytes incubated with isoproterenol

Hamster adipocytes were incubated at 37° for 16 min. Isoproterenol (1.0 μM), alone or in combination with clonidine or methoxamine, was added and the incubation was continued for an additional 4 min. Each value represents the mean ± standard error of the mean of seven experiments using methoxamine or three experiments using clonidine; each experiment was performed on a different day.

Alpha-adrenergic agent	Phosphorylase, %*a* activity (−/+ 2 mM AMP)	
	Control	Isoproterenol
None	39.9 ± 1.9	56.6 ± 1.2
Methoxamine, 10 μM	42.4 ± 2.6	61.6 ± 3.0
Δ due to methoxamine	2.5 ± 2.8	5.0 ± 3.5
None	46.6 ± 3.7	65.6 ± 2.1
Methoxamine, 100 μM	53.1 ± 3.7	69.7 ± 2.2
Δ due to methoxamine	6.2 ± 0.6[a]	4.1 ± 1.6[b]
None	54.3 ± 6.3	68.1 ± 5.4
Clonidine, 10 μM	57.5 ± 3.2	68.8 ± 4.4
Δ due to clonidine	3.2 ± 4.7	0.7 ± 3.2

[a] Significantly different from zero, $p < 0.01$.
[b] Significantly different from zero, $p < 0.05$.

Methoxamine activation of phosphorylase was unaffected by the presence of 100 μM propranolol (propranolol alone, 43.9 ± 1.8%*a*; plus methoxamine, 50.3 ± 1.5%*a*; $p < 0.05$), an observation that excludes the possibility that the response to this agent was due to interaction with *beta*-adrenergic receptors. In contrast to propranolol, the *alpha*-adrenergic blocking drugs phenoxybenzamine, yohimbine, and prazosin completely blocked methoxamine activation of phosphorylase (Table 3). Phenoxybenzamine, yohimbine, and prazosin did not influence basal phosphorylase activity. Prazosin, an $alpha_1$-adrenergic receptor antagonist (7, 19) exhibited the highest potency in inhibiting methoxamine activation of phosphorylase. This blocker inhibited the methoxamine response by 30% when present at a concentration of 0.10 μM, and fully inhibited it at 1.0 μM. Yohimbine, an $alpha_2$-site-preferential antagonist (7, 19) also blocked the methoxamine response, but a concentration of 3–10 μM was required. Phenoxybenzamine, which has been found to be more active against $alpha_1$-adrenergic receptor (3), was the least effective antagonist, not blocking methoxamine activation of phosphorylase until a concentration of 30 μM was present.

In Table 4 are presented results obtained when adipocytes were exposed to methoxamine or clonidine in combination with IBMX. As seen previously, methoxamine produced a modest increase in activity of phosphorylase *a*, whereas clonidine was without significant effect; IBMX produced the expected large activation of the enzyme. When clonidine was present along with IBMX, the response to the methyl xanthine was reduced by nearly 50%. Methoxamine similarly reduced the IBMX stimulation of phosphorylase activity, but its effect was smaller than that produced by clonidine. However, when prazosin was present, methoxamine caused a much larger decrease in phosphorylase *a* activity. As shown in Table 3, prazosin fully blocked the stimulatory effect of methoxamine on phosphorylase activity in the absence of IBMX.

The interaction between the effects of IBMX and methoxamine on phosphorylase *a* activity was studied

TABLE 3

Blockade of methoxamine activation of phosphorylase with prazosin, yohimbine, and phenoxybenzamine

Hamster adipocytes were incubated at 37° for 16 min. Methoxamine (100 μM), alone or in combination with various concentrations of prazosin, yohimbine, or phenoxybenzamine, was then added and the incubation was continued for an additional 4 min. Each value represents the mean ± standard error of the mean of the number of experiments given by the value in parentheses. Each experiment was performed on a different day.

Blocker	Control	Phosphorylase, %*a* activity (−/+ 2 mM AMP)	
		Methoxamine	Δ
None (18)	46.90 ± 1.39	51.14 ± 1.88	4.24 ± 1.28[a]
Prazosin, 0.01 μM (8)	45.02 ± 2.40	50.38 ± 1.95	5.35 ± 1.77[a]
Prazosin, 0.10 μM (8)	45.96 ± 2.01	49.37 ± 1.98	3.41 ± 0.93[a]
Prazosin, 1.0 μM (8)	46.29 ± 1.32	46.81 ± 2.29	0.52 ± 1.44
None (10)	49.6 ± 3.5	58.8 ± 4.3	9.2 ± 3.8[b]
Yohimbine, 3 μM (6)	57.4 ± 2.1	61.6 ± 2.4	4.2 ± 2.7
Yohimbine, 10 μM (10)	52.9 ± 3.8	52.7 ± 2.9	−0.2 ± 3.3
Yohimbine, 30 μM (9)	51.7 ± 2.3	51.8 ± 4.5	0.1 ± 3.5
Yohimbine, 100 μM (10)	52.9 ± 3.4	54.7 ± 3.7	1.8 ± 2.2
None (5)	46.7 ± 1.4	50.9 ± 1.7	4.2 ± 1.1[b]
Phenoxybenzamine, 10 μM (5)	45.3 ± 2.0	56.9 ± 1.8	11.5 ± 3.0[b]
Phenoxybenzamine, 30 μM (5)	49.5 ± 1.6	50.1 ± 2.8	0.6 ± 3.2
Phenoxybenzamine, 100 μM (5)	47.5 ± 3.2	47.6 ± 2.1	0.1 ± 3.7

[a] Significantly different from value obtained with no added methoxamine, $p < 0.01$.

[b] Significantly different from value obtained with no added methoxamine, $p < 0.05$.

TABLE 4

Partial inhibition of IBMX-activated phosphorylase by methoxamine and clonidine

Hamster adipocytes were incubated at 37° for 16 min. IBMX (0.10 mM), alone or in combination with methoxamine or clonidine, was added and the incubation was continued for an additional 4 min. Each value represents the mean ± standard error of the mean of the number of experiments given in parentheses. Each experiment was performed on a different day.

Alpha-adrenergic agent	Phosphorylase, %*a* activity (−/+ 2 mM AMP)		
	Control	IBMX	Δ due to IBMX
None (21)	43.9 ± 1.6	66.6 ± 2.3	+22.6 ± 1.9
Methoxamine, 10 μM (13)	46.9 ± 2.5	64.5 ± 2.6	+17.7 ± 2.1[a]
Methoxamine, 100 μM (17)	50.4 ± 1.8	64.9 ± 2.4	+14.5 ± 1.7[a]
Prazosin, 1 μM (5)	42.7 ± 1.3	63.7 ± 4.9	+21.1 ± 4.0[a]
Methoxamine, 100 μM + prazosin, 1 μM (15)	44.3 ± 2.1	48.7 ± 1.9	+4.4 ± 2.1
Clonidine, 10 μM (9)	47.0 ± 2.3	59.0 ± 2.8	+12.0 ± 1.6[a]

[a] Significantly different from value obtained with no added *alpha*-adrenergic agent, $p < 0.001$.

further using a lower concentration of IBMX. The data in Table 5 show that methoxamine completely prevented the activation of phosphorylase caused by 20 μM IBMX. This inhibitory effect of methoxamine was antagonized by the *alpha*$_2$ blocker yohimbine, but not by prazosin, *alpha*$_1$ blocker. Indeed, when yohimbine was present, the stimulatory effects of methoxamine and IBMX on phosphorylase *a* activity were approximately additive, as was seen when isoproterenol was studied (Table 2). It should be noted that the concentration of yohimbine used in this experiment is much less than that required to block methoxamine activation of phosphorylase (Table 3).

DISCUSSION

The present paper reports the first investigation of the regulation of phosphorylase activity in hamster adipocytes. Over-all, the results obtained using these cells resemble those obtained from the more commonly used rat adipocytes. Thus, agents that promote accumulation of cyclic AMP (e.g., isoproterenol, norepinephrine, ACTH, and IBMX) produce a large activation of phosphorylase. Conversely, glucose decreases phosphorylase activity. Both of these responses have been reported previously for rat adipose tissue (1, 16–18, 20). However, in contrast to rat adipocytes, where insulin decreases phosphorylase activity (2, 17, 21), in hamster adipocytes insulin has no detectable effect on phosphorylase in the presence or absence of glucose. This apparent insensitivity of hamster adipocyte phosphorylase to insulin is consistent with studies documenting a similar insensitivity of hamster adipose tissue to antilipolytic (22) and lipogenic actions of insulin (23).

In rat adipocytes the presence of an *alpha*-adrenergic component to catecholamine activation of phosphorylase was established by the work of Lawrence and Larner (1, 2). The present study documents a similar *alpha*-adrenergic response of hamster adipocytes. Adipose tissue is similar in this regard to liver, in which both *alpha*- and *beta*-adrenergic receptors are involved in phosphorylase activation (24). However, in liver, *alpha*-adrenergic stimuli produce a much greater activation of phosphorylase than do *beta*-adrenergic stimuli (24), whereas in rat and

TABLE 5

Effect of alpha-adrenergic antagonists on methoxamine inhibition of IBMX-stimulated phosphorylase

Hamster adipocytes were incubated at 37° for 16 min. IBMX (20 μM), alone or in combination with methoxamine or methoxamine plus prazosin or yohimbine, was added and the incubation was continued for an additional 4 min. Each value represents the mean ± standard error of the mean of five experiments. Each experiment was performed on a different day.

Alpha-adrenergic agents	Phosphorylase, %*a* activity (−/+ 2 mM AMP)		
	Control	IBMX	Δ due to IBMX
None	45.4 ± 4.1	55.0 ± 4.3	+9.6 ± 3.0[a]
Methoxamine, 100 μM	51.7 ± 3.7[b]	53.9 ± 4.7	+2.2 ± 1.8
Methoxamine + prazosin, 1 μM	48.1 ± 2.1	52.4 ± 3.8	+4.3 ± 2.6
Methoxamine + yohimbine, 1 μM	51.9 ± 3.7[b]	62.1 ± 4.6	+10.2 ± 1.9[b]

[a] Significantly different from zero, $p < 0.05$.

[b] Significantly different from zero, $p < 0.01$.

hamster adipose tissue, the *beta*-adrenergic mechanism elicits the greater activation of phosphorylase (1, 2). It would appear, therefore, that the *alpha*-adrenergic mechanism in adipose tissue is of minor physiological importance in comparison with the *beta*-adrenergic mechanism.

In rat adipocytes, *alpha*-adrenergic stimuli have been found only to increase phosphorylase *a* activity (1, 2). However, using hamster fat cells, we find that *alpha*-adrenergic stimuli can both increase and decrease the activation state of phosphorylase. Phosphorylase activation is observed in response to methoxamine present by itself or in combination with isoproterenol. Phosphorylase inactivation, on the other hand, is seen in response to clonidine and, to a smaller extent, methoxamine when either agonist is present in combination with IBMX. We attribute these oppositely directed responses to the presence of two independent *alpha*-receptors on hamster adipocytes. Pharmacological and radioligand binding studies on isolated hepatocytes indicate that *alpha*-adrenergic activation of phosphorylase is mediated by *alpha*$_1$-receptors (8, 25, 26), and it would seem likely that a similar situation exists on adipocytes. The observation that methoxamine, an *alpha*$_1$-preferential agonist (3–5), activates phosphorylase whereas clonidine, an *alpha*$_2$-preferential agonist (3–5), does not is consistent with involvement of *alpha*$_1$-receptors in this response. Similarly, the finding that prazosin was the most potent blocker of methoxamine activation of phosphorylase is also consistent with involvement of *alpha*$_1$-receptors. Although methoxamine activation of phosphorylase was blocked by yohimbine, *alpha*$_2$-selective antagonist, it should be noted that the apparent sensitivity of the adipocyte enzyme to yohimbine is nearly equal to the sensitivity of *alpha*-adrenergic-activated phosphorylase in hepatocytes to this antagonist (8, 25). Two studies (13, 27) employing radioligand binding techniques have shown the presence of a small population of *alpha*$_1$-receptors on hamster adipocytes. However, in contrast to hepatocytes, where prazosin was found to be 1000-fold more potent than yohimbine at blocking *alpha*-adrenergic activation of phosphorylase (25) in adipocytes, prazosin was only 10-fold more effective than yohimbine. Moreover, the concentration of methoxamine required to activate phosphorylase is high in comparison with concentrations used to activate other *alpha*$_1$-receptor-mediated responses. For example, Stark *et al.* (4) calculated an ED_{20} of 7.4×10^{-7} M for methoxamine-stimulated contraction of rabbit pulmonary artery. In contrast, the lowest effective methoxamine concentration which activates adipocyte phosphorylase is 10^{-5} M. Taken together, these data indicate that the hamster adipocyte *alpha*-receptor mediating phosphorylase activation has similarities to other *alpha*$_1$-adrenergic receptors with regard to its relative affinities for the antagonists prazosin and yohimbine and the agonists methoxamine and clonidine. However, the adipocyte *alpha*-receptor has much lower apparent affinities for prazosin and methoxamine than other *alpha*$_1$-adrenergic receptors.

Previous studies from our laboratory (9, 11, 28) and others (12, 13) have documented clonidine inhibition of lipolysis and cyclic AMP accumulation in hamster adipocytes that is mediated by *alpha*$_2$-adrenergic receptors. Several of these same studies (11, 28) reported that clonidine is a more effective inhibitor in the presence of IBMX than is isoproterenol. The present observations showing clonidine inhibition of IBMX-stimulated, but not isoproterenol-stimulated, phosphorylase are consistent, therefore, with our previous findings on clonidine inhibition of lipolysis and cyclic AMP levels and support the view that inhibition of IBMX-activated phosphorylase is mediated by *alpha*$_2$-receptors.

Methoxamine has similarly been found to inhibit IBMX-stimulated lipolysis (11, 28), and the present report shows methoxamine inhibition of IBMX-activated phosphorylase. Thus, methoxamine can inhibit as well as activate phosphorylase. These oppositely directed responses to methoxamine most likely reflect the interaction of methoxamine with either *alpha*$_1$-adrenergic receptors leading to phosphorylase activation or *alpha*$_2$-adrenergic receptors leading to inactivation of phosphorylase. It follows, therefore, that the response of hamster adipocytes to methoxamine represents a summation of its two opposing influences. In direct support of this contention is the finding that methoxamine inhibition of IBMX-activated phosphorylase is markedly enhanced by the *alpha*$_1$ blocker prazosin, which blocks the stimulatory effect of methoxamine. Conversely, the *alpha*$_2$ blocker yohimbine at 1 μM blocks the inhibitory effect of methoxamine while leaving unaltered the stimulatory effect of the *alpha*-agonist.

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