# DECREASED SENSITIVITY TO $\alpha_2$ ADRENERGIC AMINES, ADENOSINE AND PROSTAGLANDINS IN WHITE FAT CELLS FROM HAMSTERS TREATED WITH PERTUSSIS VACCINE

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## 1. Introduction

The release of insulin from pancreatic islets is inhibited by α-adrenergic activation and is increased by stimulation of β-adrenoceptors [1]. α-Adrenoceptors have been divided into 2 subtypes, $\alpha_1$ and $\alpha_2$, by their affinities for agonists and antagonists [2] and their underlying mechanism of signal transduction [3]. The α-adrenoceptor involved in the regulation of plasma insulin level is of the $\alpha_2$ subtype [4]. $\alpha_2$-Adrenoceptors are linked in an inhibitory fashion to adenylate cyclase [3,5,6].

The decrease in blood insulin level due to α-adrenoceptor activation is effectively blocked by pertussis vaccination in rats [7]. This effect of the vaccine seems to be due to a protein produced by *Bordetella pertussis* [8,9]. Hamster adipocytes have $\alpha_2$-adrenergic receptors linked in an inhibitory fashion to adenylate cyclase [3,5]. Activation of these receptors decreases basal and stimulated cyclic AMP levels and lipolysis [3,5,10]. The possibility that administration of pertussis vaccine to hamsters may alter the sensitivity of their adipocytes to $\alpha_2$-adrenergic amines was studied. The results show that administration of pertussis vaccine to hamsters markedly decreases the sensitivity of their adipocytes to $\alpha_2$-catecholamines. Furthermore, it was found that such decrease in sensitivity is not exclusive to $\alpha_2$-adrenergic amines but it is common to other agents such as adenosine and prostaglandins.

## 2. Materials and methods

Epinephrine, isoproterenol, theophylline and prostaglandin $E_2$ ($PGE_2$) were obtained from Sigma Chemical Co. $N^6$-(L-2-phenyl-isopropyl)-adenosine (PIA), adenosine deaminase, glycerokinase, glycerophosphate dehydrogenase and $NAD^+$ were obtained from Boehringer-Mannheim. Collagenase from *Clostridium histolyticum* (lot 40C190) and bovine serum albumin (fraction V) (lot U13707) were obtained from Worthington and Armour Pharmaceutical Co., respectively. Clonidine was a generous gift of Boehringer-Ingelheim. Pertussis vaccine (strain 509, lot 29-090) was obtained from the National Institute of Hygiene (Secretaría de Salubridad y Asistencia, México).

Golden hamsters were fed ad libitum. Pertussis vaccine was injected intraperitoneally ($\sim 10^{11}$ organisms). Animals were used 3–9 day after a single injection. White adipocytes were isolated and incubated as in [5,10]. Cyclic AMP accumulation and glycerol release were determined as in [5,10].

## 3. Results

The level of cyclic AMP in the absence of hormones was slightly higher in adipocytes from pertussis-treated hamsters than in control adipocytes (42 ± 10 compared to 29 ± 2 pmol/$10^6$ cells, respectively; means ± SEM of 6 determinations in duplicate in each case). Isoproterenol, a pure β-adrenergic agonist, produced a dose-dependent increase in the level of cyclic AMP (fig.1). The maximal accumulation of the cyclic nucleotide was not affected in adipocytes from pertussis-treated hamsters. However, a decreased accumulation of cyclic AMP in response to the lowest dose of isoproterenol used ($10^{-8}$ M) was observed in fat cells from pertussis-sensitized hamsters (fig.1). Epinephrine, an α–β-adrenergic agonist, produced a

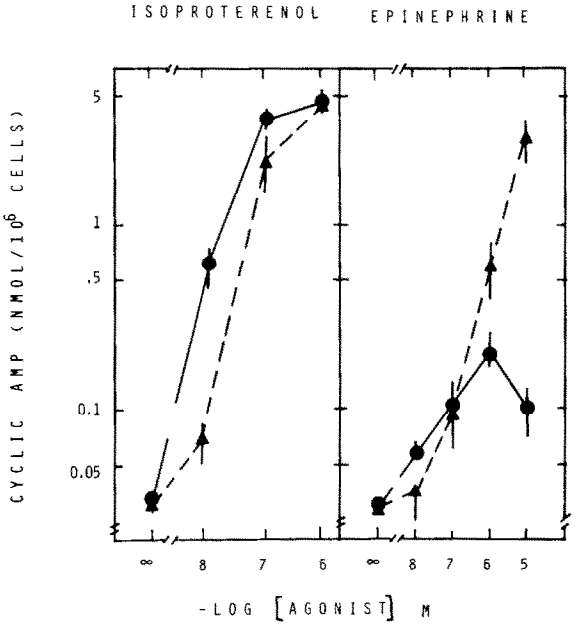


Fig.1. Dose–response curves for the effects of isoproterenol or epinephrine on the accumulation of cyclic AMP in adipocytes from hamsters. Fat cells ($1-2 \times 10^5$) were incubated for 10 min in 1 ml Krebs-Ringer phosphate buffer containing 3% albumin, 0.5 μg adenosine deaminase, 100 μM theophylline and different concentrations of either isoproterenol or epinephrine. (●) Adipocytes from control hamsters; (▲) adipocytes from hamsters treated with pertussis vaccine. Results are the means and vertical lines represent the SEM of 6 expt performed in duplicate. Results are plotted on a log scale.

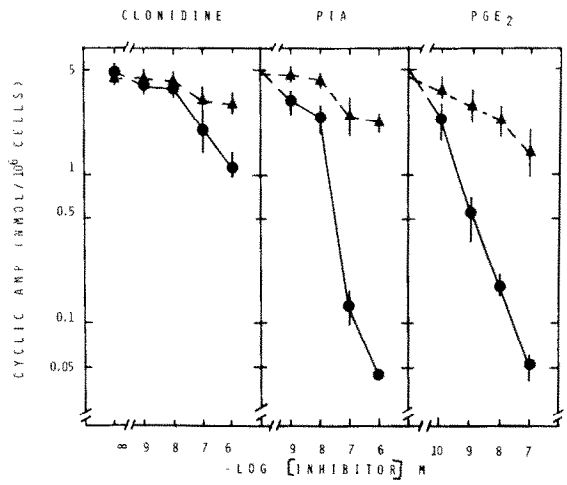


Fig.2. Dose–response curves for the effects of clonidine, $N^6$-(2-phenyl-isopropyl) adenosine (PIA) and prostaglandin $E_2$ ($PGE_2$) on the accumulation of cyclic AMP due to isoproterenol in adipocytes from hamsters. Fat cells ($1-2 \times 10^5$) were incubated for 10 min in 1 ml Krebs-Ringer phosphate buffer containing 3% albumin, 0.5 μg adenosine deaminase, 100 μM theophylline, 10 μM isoproterenol and different concentrations of clonidine, PIA or $PGE_2$. (●) Adipocytes from control hamsters; (▲) adipocytes from hamsters treated with pertussis vaccine. Results are the means and vertical lines represent the SEM of 6 expt performed in duplicate. Results are plotted on a log scale.

dose-dependent accumulation of cyclic AMP in control adipocytes (fig.1). However, the maximal accumulation was much smaller than that due to isoproterenol (~10-fold increase compared to ~150-fold increase, respectively). This is due to inhibition of adenylate cyclase by activation of $\alpha_2$-adrenoceptors [3,5,6]. In adipocytes from pertussis-treated hamsters the accumulation of cyclic AMP due to epinephrine was much higher, reaching levels similar to those obtained with isoproterenol (fig.1). The data suggest that in adipocytes from hamsters treated with pertussis vaccine, there is a decreased $\alpha_2$-adrenergic sensitivity.

To further test this point, the ability of the $\alpha_2$-adrenergic agonist, clonidine, to decrease the level of cyclic AMP due to isoproterenol was assayed. The sensitivity to clonidine was significantly decreased in adipocytes from treated animals (fig.2). In order to determine if the action of pertussis vaccine was exclusively on $\alpha_2$-adrenoceptors, the ability of other agents, which inhibit adenylate cyclase, to decrease the levels of cyclic AMP due to isoproterenol was tested. PIA and $PGE_2$ decreased the accumulation of cyclic AMP due to β-adrenergic stimulation to a much lesser extent in adipocytes from treated hamsters than in control adipocytes (fig.2).

Basal lipolysis (in the absence of adenosine deaminase and theophylline) was higher in adipocytes from treated hamsters than in the control adipocytes (0.80 ± 0.02 and 0.33 ± 0.01 μmol glycerol/$10^6$ cells in 60 min incubation, respectively; results are the means ± SEM of 4 expt in each case). However, lipolysis due to adenosine deaminase plus theophylline was only slightly higher in adipocytes from treated hamsters than in control fat cells (fig.3). This suggests that in fat cells from treated hamsters the sensitivity to endogenously released adenosine was significantly reduced. However, the possibility that the sensitivity to another endogenous regulator(s) may also be decreased can not be ruled out. The ability of clonidine, PIA and $PGE_2$ to inhibit lipolysis due to adenosine deaminase plus theophylline was also significantly reduced in pertussis-sensitized hamsters (fig.3).

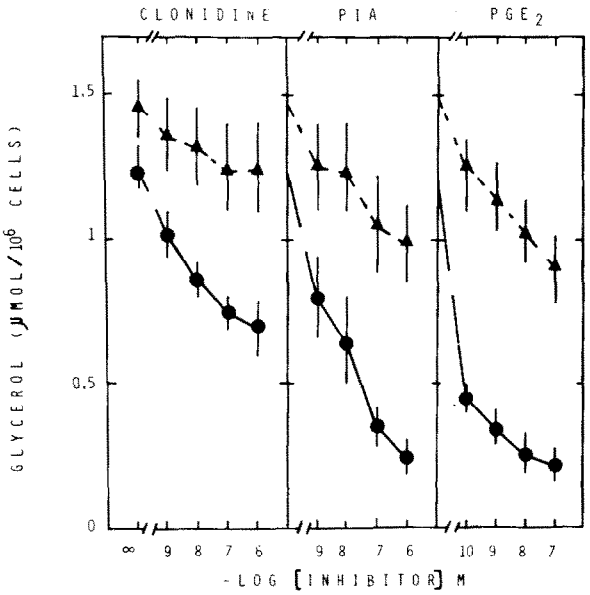


Fig.3. Dose–response curves for the effects of clonidine, $N^6$-(2-phenyl-isopropyl) adenosine (PIA) and prostaglandin $E_2$ ($PGE_2$) on lipolysis due to adenosine deaminase and theophylline. Fat cells ($1-2 \times 10^5$) were incubated for 60 min in 1 ml Krebs-Ringer phosphate buffer containing 3% albumin, 0.5 μg adenosine deaminase, 100 μM theophylline and different concentrations of clonidine, PIA or $PGE_2$. (•) Adipocytes from control hamsters; (▲) adipocytes from hamsters treated with pertussis vaccine. Results are the means and vertical lines represent the SEM of 8 expt.

## 4. Discussion

Administration of pertussis vaccine to hamsters produced a marked decrease in the sensitivity of their adipocytes to $\alpha_2$-adrenergic amines, PIA and $PGE_2$ as reflected by both cyclic AMP levels and lipolysis. $\alpha_2$-Adrenergic amines, PIA, and $PGE_2$ share the property of inhibiting adenylate cyclase through receptor-mediated processes. Inhibition of adenylate cyclase by hormones and neurotransmitters seems to involve at least 3 molecular entities: the catalytic subunit of adenylate cyclase; the receptor; and a guanine nucleotide binding protein [11,12]. Calcium does not seem to be involved in inhibition of adenylate cyclase by these agents.

It is unlikely that treatment with pertussis vaccine may decrease the level of receptors for $\alpha_2$-adrenergic amines, adenosine and prostaglandins. Therefore, it is possible that the action of pertussis vaccine may occur at the level of the coupling between the receptor and the cyclase.

These data are consistent with the data in [7–9] where pertussis sensitization or administration of the purified 'islet-activating protein' abolished the ability of α-adrenergic agents and somatostatin to inhibit glucose-induced insulin release, cyclic AMP accumulation and $^{45}Ca^{2+}$ uptake by pancreatic islets from rats. It was concluded that the action of the 'islet-activating protein' is the result of sustained activation of native calcium ionophores present on the cell membrane [8]. The present findings suggest a complementary or alternative explanation:

> It is possible that the 'islet-activating protein', present in the vaccine, may block the transfer of inhibitory information from the receptor to adenylate cyclase.

Calcium and cyclic AMP are known to play important roles as a link in the stimulus–secretion coupling in the pancreatic β-cell [13]. Changes in the level of cyclic AMP may explain the altered calcium fluxes and insulin secretion due to epinephrine in islets from rats treated with the 'islet-activating protein'.

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