An Adrenergic Receptor Mechanism for the Control of Cyclic 3'5' Adenosine Monophosphate Synthesis in Tissues*

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Received July 31, 1967

The tissue response to catecholamines depends on the presence of adrenergic receptor sites, classified as alpha and beta on the basis of their response to sympathomimetic amines and adrenergic blocking agents. (Ahlquist, 1948). Murad et al. (1963) have reported that epinephrine stimulates the synthesis of cyclic 3'5' adenosine monophosphate (cyclic 3'5' AMP) in heart muscle by a direct action on adenyl cyclase, and that this stimulatory effect is prevented by beta adrenergic blockade. Similar findings have been reported in other tissues (Sutherland and Robinson, 1966). Studies in our laboratory have shown that the effects of epinephrine on insulin secretion in the rat are mediated via alpha and beta adrenergic receptor sites in the islet. Stimulation of the beta adrenergic receptor increases, and stimulation of the alpha adrenergic receptor inhibits insulin secretion, suggesting that these receptors mediate divergent effects of epinephrine on cyclic 3'5' AMP synthesis (Turtle et al, 1967). Recently it has been reported that the metabolic effects of epinephrine on the rat epididymal fat pad (Butcher et al, 1965) and the toad bladder (Handler et al, 1967) are also differentially affected by alpha and beta receptor blockade.

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* Supported in part by U.S.P.H.S. Grant AM-01921

[1] Fellow in Metabolism, supported by the Royal Australasian College of Physicians

We have examined the effects of epinephrine, in the presence and absence of alpha and beta adrenergic blocking agents, on the cyclic 3'5' AMP concentration and correlated these changes with the metabolic response pattern in three tissues - isolated pancreatic islets, isolated fat cells and toad bladders. Evidence is presented that stimulation of the alpha adrenergic receptor decreases cyclic 3'5' AMP synthesis, whereas stimulation of the beta adrenergic receptor produces an increase in the cyclic 3'5' AMP level. Furthermore, the pattern of metabolic response in these tissues correlates with the cyclic 3'5' AMP concentration, and is reproduced by the addition of the cyclic nucleotide in vitro (Sussman et al, 1966, Rizack, 1964, Orloff and Hanlder, 1962).

Experimental procedure. Alpha adrenergic blockade was maintained with phentolamine (Ciba) and beta adrenergic blockade with propranolol (Ayerst).

Isolated rat pancreatic islets were prepared by the method of Lacy (1967). Deoxyribonucleic acid in the islets was estimated by the fluorometric procedure of Kissane and Robins (1958).

Isolated rat fat cells were prepared by the method of Rodbell (1964). Cell counts were performed in a Neubauer hemocytometer chamber. Lipolysis was measured by determining the rate of free fatty acid release. Free fatty acids were extracted by the procedure of Dole (1956) and determine colorimetrically (Duncombe, 1963).

Bilobed toad bladders were removed from pithed Bufo marinus toads and preincubated in Bentley's buffer for 10 minutes before use. (Bentley, 1958).

The cyclic 3'5' AMP concentration in the tissues was measured by the method of Turtle and Kipnis (1967).

Results and Discussion

I. Isolated Pancreatic Islets (Table I). Theophylline (180 +g/ml), a potent inhibitor of cyclic 3'5' nucleotide phosphodiesterase (Butcher and Sutherland, 1962), produced a 1500 per cent increase in the cyclic nucleotide level from 355 $\pm$ 50 +moles/g DNA to 5570 $\pm$ 302 μmoles/g DNA. Epinephrine (10 μg/ml) almost completely inhibited this effect (415 $\pm$ 12 μmoles/g DNA.) This

action of epinephrine reflected alpha adrenergic receptor stimulation since it was completely abolished by phentolamine. When residual beta adrenergic receptor activity was blocked with propranolol, the addition of epinephrine produced cyclic 3'5' AMP levels less than those in the control. Glucagon (1 μg/ml) also stimulated the accumulation of cyclic 3'5' AMP. These changes in pancreatic islet cyclic 3'5' AMP correlate well with the pattern of insulin release observed in vivo in the rat in response to theophylline, epinephrine and adrenergic blocking agents (Turtle and Kipnis, 1967).

Table I

The effects of theophylline, glucagon, epinephrine and adrenergic blocking agents on pancreatic islet cyclic 3'5' AMP content and insulin release in the rat.

Experimental conditions	Theophylline	Cyclic 3'5' AMP concentration in pancreatic islets μmoles/g	Insulin secretion in vivo μunits-minutes ml
Control	-	355 ± 50	0
	+	5,570 ± 302	487 ± 42
Epinephrine	+	415 ± 12	208 ± 29
Epinephrine + phentolamine	+	4,450 ± 363	1,272 ± 36
Epinephrine + propranolol	+	305 ± 20	0
Glucagon	+	11,950 ± 431	1,023 ± 97

Isolated rat islets prepared from 6 male Charles River rats weighing 500-600 g were incubated for 1 hour at 37°C in vials containing 25-50 islets in 2 ml of albumin-bicarbonate buffer pH 7.4 and 5.6 mM glucose. Epinephrine (10 μg/ml), phentolamine (10 μg/ml), glucagon (1 μg/ml) and theophylline (180 μg/ml) were added as indicated in the table. The cyclic 3'5' AMP concentration was measured in duplicate on 200-300 islets obtained by pooling the contents of 8 incubation vials. The insulin secretion in vivo in the rat was previously reported from this laboratory and represents the area circumscribed by the plasma insulin response curve. (Turtle et al, 1967.)

II. Isolated Fat Cells (Table II). In the presence of epinephrine (0.05 μg/ml), cyclic 3'5' AMP increased fourfold from 23 ± 3 μmoles/10^{12} cells to 95 ± 7 μmoles/10^{12} cells, and lipolysis was correspondingly

accelerated. Lipolysis was slightly accelerated by propranolol (5 μg/ml) from 11.4 ± 0.8 mmoles/10^{12} cells/30 minutes to 15.0 ± 0.5 mmoles/10^{12} cells/30 minutes. In the presence of propranolol, epinephrine (0.05 μg/ml) decreased the cyclic 3'5' AMP concentration to 13 ± 2 μmoles/10^{12} cells, a level below that in the control. There was a corresponding inhibition in the release of free fatty acids to 70 percent of the control level, and 50 percent of the level in the presence of propranolol alone. When the effect of epinephrine on the alpha adrenergic receptor was blocked with phentolamine (5 μg/ml), cyclic 3'5' AMP levels (70 ± 8 μmoles/10^{12} cells) comparable to those with epinephrine alone (95 ± 5 μmoles/10^{12} cells) as well as corresponding rates of free fatty acid release were observed.

Table II

The effects of epinephrine and adrenergic blocking agents on the cyclic 3'5' AMP concentration and the rate of lipolysis in isolated fat cells.

Experimental conditions	Cyclic 3'5' AMP concentration μmoles/10^{12} cells	FFA release mmoles/10^{12} cells/30 minutes
Control	23 ± 3	11.4 ± 0.8
Epinephrine	95 ± 5	200
Epinephrine + propranolol	13 ± 2	8.0 ± 0.2
Propranolol	-	15.0 ± 0.5
Epinephrine + phentolamine	70 ± 8	200
Phentolamine	-	13.1 ± 1.1

Isolated fat cells prepared from 8 male Charles River rats, were incubated in 1 ml of albumin-bicarbonate buffer pH 7.4 containing 2% bovine serum albumin and 3 mM glucose for 30 minutes at 37°C. Epinephrine (0.05 μg/ml), propranolol (5 μg/ml) and phentolamine (5 μg/ml) were added as indicated in the table. Cell counts ranged from 2.8 to 3.1 x 10^6 cells/ml. The cyclic 3'5' AMP concentration was determined on approximately 3 x 10^6 cells. Each value represents the mean ± S.E.M. of 4 determinations.

III. Toad Bladders (Table III). Theophylline (180 μg/ml) increased cyclic 3'5' AMP from 1.40 ± 0.07 mμmoles/g to 9.30 ± 0.5 mμmoles/g, an increase of over 600 per cent. These values are higher than the levels

reported by Handler et al (1965), who found a cyclic 3'5' AMP concentration of 0.45 mμmoles/g which increased 170 per cent in the presence of theophylline (1.8 mg/ml). These workers, however, preincubated the bladders for 20-120 minutes, a manouver known to decrease the cyclic 3'5' AMP concentration (Handler et al, 1965). Epinephrine (1 μg/ml) alone did not significantly change the cyclic 3'5' AMP concentration. However, in the presence of theophylline (180 μg/ml), epinephrine decreased the cyclic 3'5' AMP concentration from 9.30 ± 0.5 mμmoles/g to 2.38 ± 0.3 mμmoles/g. Furthermore this inhibition of cyclic 3'5' AMP accumulation by epinephrine was completely abolished by alpha adrenergic receptor blockade with phentolamine (10 μg/ml). Under these conditions, stimulation of the beta adrenergic receptor increased the cyclic 3'5' AMP concentration 920 per cent to 21.95 ± 3.6 mμmoles/g. These changes in the cyclic nucleotide correlate well with the changes produced by theophylline, epinephrine and adrenergic

Table III

The effects of epinephrine, theophylline and phentolamine on the cyclic 3'5' AMP concentration in toad bladders.

Experimental conditions	Theophylline	Cyclic 3'5' AMP concentration	Permeability to water
		mμmoles/g	% of control
Control	-	1.40 ± 0.07	100
	+	9.30 ± 0.5	+950
Epinephrine	-	2.0 ± 0.1	-78
	+	2.38 ± 0.3	-
Epinephrine + phentolamine	+	21.95 ± 3.6	+47*

Toad bladders were incubated for 30 minutes in 20 ml Bentley's buffer pH 8.0 containing 5.6 mM glucose. Epinephrine (1 μg/ml) , phentolamine (10 μg/ml) and theophylline (180 μg/ml) were added to the incubation medium as indicated in the table. Each incubation vial contained 170-270 mg of tissue on which the cyclic 3'5' AMP was determined. Each value represents the mean ± S.E.M. of 4 determinations. The data on permeability to water were taken from Orloff and Handler (1962) and Handler et al (1967).
*In these studies theophylline was not used with the combination of epinephrine and blocking agent.

blocking agents on the permeability to water in the toad bladder, reported by Orloff and Handler (1962) and Handler et al (1967).

These data indicate that the effects of epinephrine on cyclic 3'5' AMP accumulation are mediated via alpha and beta adrenergic receptor sites. Stimulation of the alpha adrenergic receptor inhibits, and stimulation of the beta adrenergic receptor increases cyclic 3'5' AMP synthesis. In all these tissues, the effects of cyclic 3'5' AMP reproduce the effects of epinephrine when alpha adrenergic receptor activity is blocked (Sussman et al, 1966, Rizack 1964, Orloff and Handler 1962).

References

Ahlquist, R. P. (1948), Am. J. Physiol. 153, 586
Bentley, P. J. (1958), J. Endocrinol. 17, 201
Butcher, R. W., Ho, R. J., Meng, H. C. and Sutherland, E. W. (1965) J. Biol. Chem. 240, 4515
Butcher, R. W. and Sutherland, E. W. (1962), J. Biol. Chem. 237, 1244
Dole, V. P. (1956), J. clin. Invest. 35, 150
Duncombe, W. G. (1963), Biochem. J. 88, 7
Handler, J. S., Bensinger, R. and Orloff, J. (1967), J. clin. Invest. 46, 1066
Handler, J. S., Butcher, R. W., Sutherland, E. W. and Orloff, J. (1965) J. Biol. Chem. 240, 4524
Kissane, J. M. and Robins, E. (1958), J. Biol. Chem. 233, 184
Lacy, P. and Kostianovsky, M. (1967), Diabetes 16, 35
Murad, Y.-M., Rall, T. W. and Sutherland, E. W. (1962), J. Biol. Chem. 237, 1233
Orloff, J. and Handler, J. S. (1962), J. clin. Invest. 41, 702
Rizack, M. A. (1964), Ann. N. Y. Acad. Sci. 131, 250
Rodbell, M. (1964), J. Biol. Chem. 239, 375
Sussman, K. E., Vaughn, G.D. and Timmer, R. F. (1966), Program of the 26th meeting of the American Diabetes Association, Abstract 28
Sutherland, E. W. and Robison, G. A. (1966), Pharmacol. Rev. 18, 145
Turtle, J. R. and Kipnis, D. M. (1967) Biochemistry - submitted for publication
Turtle, J. R., Littleton, G. K. and Kipnis, D. M. (1967), Nature 213, 727