Mechanism of Epinephrine and Serotonin Inhibition of Insulin Release in the Golden Hamster in Vitro

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SUMMARY

Similarities and differences in the mechanisms by which epinephrine and serotonin inhibit insulin secretion were studied in an in vitro golden hamster pancreas system. Both amines were shown to inhibit the insulin release stimulated by either high glucose (3 mg./ml.) or dibutyryl cyclic AMP 1 mg./ml. The beta adrenergic blocking agent propranolol had no effect on either basal or high glucose-stimulated insulin release. It also was without effect on the serotonin inhibition of high glucose-stimulated insulin release. Phentolamine, an alpha adrenergic blocking agent, prevented the serotonin inhibition of high glucose or dibutyryl cyclic AMP mediated insulin release. Phentolamine also blocked the action of epinephrine in inhibiting dibutyryl cyclic AMP mediated insulin release. The data indicate that both amines block insulin release by interfering with the action of 3′5′ cyclic AMP in causing insulin release. Phentolamine appears to act at the same locus, i.e., in the action of 3′5′ cyclic AMP. The only difference noted between serotonin and epinephrine action on the pancreatic beta cell was that methysergide maleate, a known serotonin antagonist, was able to block serotonin action but not that of epinephrine. DIABETES *19*:480-86, July, 1970.

Many investigators have presented evidence to suggest that an adrenergic mechanism is responsible for the control of insulin secretion from the pancreatic beta cell. Epinephrine and norepinephrine inhibit insulin secretion in response to glucose,[1-3] tolbutamide[2,3] or glucagon,[2] and this inhibition is prevented by α adrenergic blocking agents.[1,4] β adrenergic agents, such as isoproterenol, stimulate insulin release and this is inhibited by β adrenergic blocking agents.[4,5] Measurements of 3′5′ cyclic AMP levels in isolated islets incubated with various adrenergic agents, and α and β adrenergic blocking agents, have suggested a unifying concept involving the adenyl cyclase system to explain these findings.[6,7] This theory postulates that insulin secretion results from stimulation of the β adrenergic receptor which activates the adenyl cyclase system, resulting in increased production of 3′5′ cyclic AMP which is responsible for insulin secretion. Stimulation of the α adrenergic system inhibits 3′5′ cyclic AMP generation and thus prevents increased insulin secretion in response to insulinogenic stimuli.

During a series of studies on the effect of serotonin on insulin secretion, it was noted that this amine probably inhibits insulin secretion by directly interfering with the action of 3′5′ cyclic AMP.[8] The present report examines the similarities and differences in the mode of action of serotonin and epinephrine in inhibiting insulin release. The data indicate that both of these monoamines act by inhibiting the stimulatory effects of 3′5′ cyclic AMP on insulin release. This inhibitory effect is prevented by the α adrenergic blocking agent phentolamine.

MATERIAL AND METHODS

A golden hamster in vitro pancreas system previously described was used in the present studies.[8] In this system each piece of pancreas undergoes two sequential fifteen-minute incubation periods. The first incubation is carried out in basal media (initial incubation) while the second incubation is carried out in media containing the test substance added to basal media (treatment incubation). The basal media is Krebs Ringer bicarbonate buffer with 0.005 M pyruvate, 0.005 M fumarate, 0.005 M glutamate, 60 mg./100 ml. glucose and 400 mg./100 ml. bovine serum albumin. At the conclusion of each incubation, aliquots from the incubation media are placed in chilled tubes and directly assayed for insulin by radioimmunoassay technic. Net insulin release is expressed as the difference in insulin release between the treatment and initial incubations (μU./mg. pancreas/15 min.). In a series of studies carried out in which the pancreas pieces were incubated in basal

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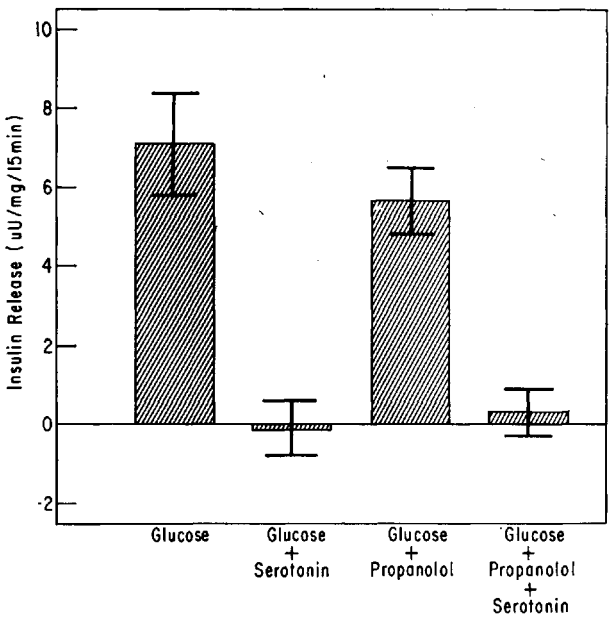

FIG. 1. Effect of propranolol on glucose (3 mg./ml.) mediated insulin secretion and the inhibition of this secretion by serotonin. Serotonin inhibits glucose-mediated insulin secretion ($p < .01$) while propranolol neither blocks glucose-mediated insulin release, nor prevents the inhibition of this release by serotonin. The bars represent the means and the brackets the S.E.M. of ten observations. Serotonin and propranolol were present at 10^{-4} M.

media in two consecutive incubation periods, it was noted that the insulin release in the second incubation differed from that in the first incubation by only —10.7 per cent (standard deviation ±25.0 per cent). This demonstrates the reproducibility of insulin secretion in the two consecutive periods, with the insulin release in the second period usually being slightly less than that in the first, thus resulting in a slight negative net insulin release.

Serotonin creatinine sulfate and N^6, $o^{2'}$-dibutyryl adenosine-3′5′ cyclic phosphate (DB-AMP) were purchased from Cal Biochemical Company, DL-propranolol (Inderal) was purchased from Ayerst Laboratories, and epinephrine was purchased from Parke, Davis and Company. Phentolamine mesylate (Regitine) was a gift of CIBA Pharmaceuticals. Methysergide maleate (Sansert) was a gift from Sandoz Pharmaceuticals.

RESULTS

Effect of adrenergic blocking agents on serotonin inhibition of insulin release

Though the initial data[8] on the serotonin inhibition of DB cyclic AMP mediated insulin release suggested that serotonin must act beyond the presumed adrenergic receptor sites, studies were carried out with the β receptor blocking agent, propranolol, and the α receptor blocking agent phentolamine to further test this thesis. Figure 1 illustrates the insulin release provoked by 3 mg./ml. of glucose and the striking inhibition of this insulin release produced by serotonin. Propranolol (10^{-4} M) did not interfere with this glucose-mediated insulin release. Propranolol also did not effect the inhibitory action of serotonin on glucose-mediated insulin release.

Since in vivo studies by other investigators had suggested that propranolol blocks both glucose-mediated insulin release,[9,10] and the insulin release stimulated by tolbutamide, ACTH, glucagon and cyclic 3′5′ AMP,[9] additional studies were carried out with even higher concentrations (5×10^{-4} M) of propranolol. The results were again similar to those noted in figure 1.

Studies were then conducted to determine if pro-

TABLE 1

Effect of propranolol on in vitro insulin release from pieces of golden hamster pancreas. Propranolol was present in both initial and treatment incubations.

	Group 1	Group 2	Group 3	Group 4
Initial incubation	Basal media	Basal media	Basal media + propranolol 10^{-4} M	Basal media + propranolol 10^{-4} M
Treatment incubation	Glucose 3 mg./ml.	Glucose 3 mg./ml. + serotonin 10^{-4} M	Glucose 3 mg./ml. + propranolol 10^{-4} M	Glucose 3 mg./ml. + propranolol 10^{-4} M + serotonin 10^{-4} M
Insulin release	12.2±1.28	2.4±1.04*	12.1±2.63	4.0±1.90†

*Groups 1 and 2 are significantly different ($p < .01$).
†Groups 3 and 4 are significantly different ($p < .01$).
Data are expressed as mean ± S.E.M.

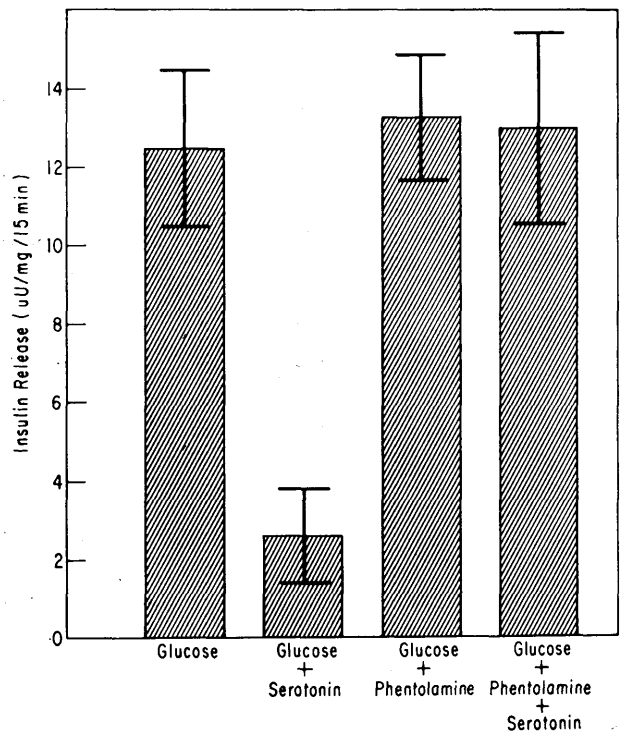

FIG. 2. Effect of phentolamine on glucose (3 mg./ml.) mediated insulin secretion. Serotonin inhibits glucose-mediated insulin secretion ($p < .01$) while phentolamine does not alter this type of insulin secretion. However, phentolamine completely blocks the inhibitory effect of serotonin on glucose-mediated insulin release. The bars represent the means and the brackets the S.E.M. of fifteen experimental observations. Serotonin and phentolamine were present at 10^{-4} M.

pranolol would alter insulin secretion induced by glucose, or prevent the inhibition of insulin secretion by serotonin if the tissues were preincubated with propranolol prior to the treatment incubation. Therefore, in addition to its presence in the treatment incubation, propranolol was also added to the initial fifteen-minute incubation period. Even under these conditions propranolol had no effect on either glucose-mediated insulin release or the inhibition of this release by serotonin (table 1).

Finally, experiments were done to determine if the inhibition of insulin release by serotonin was mediated by the α adrenergic receptors. Figure 2 illustrates the insulin release stimulated by 3 mg./ml. of glucose, and the inhibition of this insulin release by serotonin. Phentolamine did not alter glucose-mediated insulin release. In contrast to the findings with propranolol, phentolamine prevented the inhibitory effect of serotonin on glucose-mediated insulin release.

Effect of phentolamine on serotonin inhibition of DB-AMP mediated insulin release

The prevention of the serotonin inhibition of glucose-mediated insulin release by phentolamine was confusing, as previous studies had shown that serotonin blocks the stimulation of insulin release by DB-cyclic AMP,[8] while phentolamine is believed to act at an earlier step (the α adrenergic receptor site).[6] To evaluate the possibility that phentolamine may act not on the generation of 3′5′ cyclic AMP, but rather on the action of 3′5′ cyclic AMP, the studies illustrated in figure 3 were carried out. It is again noted that serotonin prevented the stimulation of insulin release by DB-AMP. This inhibition of DB-AMP mediated insulin release

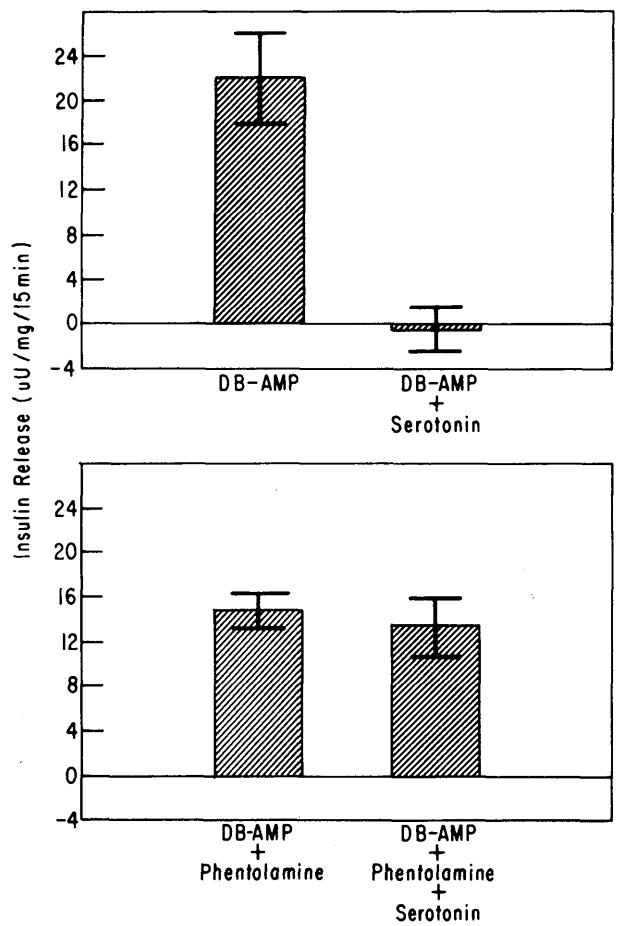

FIG. 3. Effect of phentolamine on both DB-AMP mediated insulin secretion, and the inhibition of DB-AMP secretion by serotonin. Experiment one demonstrates that serotonin inhibits DB-AMP mediated insulin secretion ($p < .01$). Experiment two demonstrates that this inhibition is prevented by phentolamine. The bars represent the means and the brackets the S.E.M. of five observations. Serotonin and phentolamine were present at 10^{-4} M while DB-cyclic AMP was present in concentrations of 1 mg./ml.

was completely blocked by phentolamine. Other studies demonstrated that phentolamine alone did not alter the insulin release stimulated by DB-AMP (1 mg./ml.). Thus phentolamine exerts an action on insulin release by interacting with serotonin in influencing the action of 3′5′ cyclic AMP.

Site of action of epinephrine on insulin release

It is believed that epinephrine impairs insulin secretion by stimulating an α adrenergic receptor. However, in view of the many physiological similarities of epinephrine and serotonin, the thesis was investigated that epinephrine also acts by inhibiting DB-cyclic AMP action. The data in figure 4 substantiate this, for they demonstrate that the marked stimulation of insulin release by DB-AMP can be blocked by epinephrine just as it was blocked by serotonin.

Further studies shown in table 2 demonstrate that the inhibition of DB-AMP mediated insulin release by epinephrine can also be prevented by phentolamine, similar to the way the inhibition by serotonin could be prevented by this α adrenergic blocking agent.

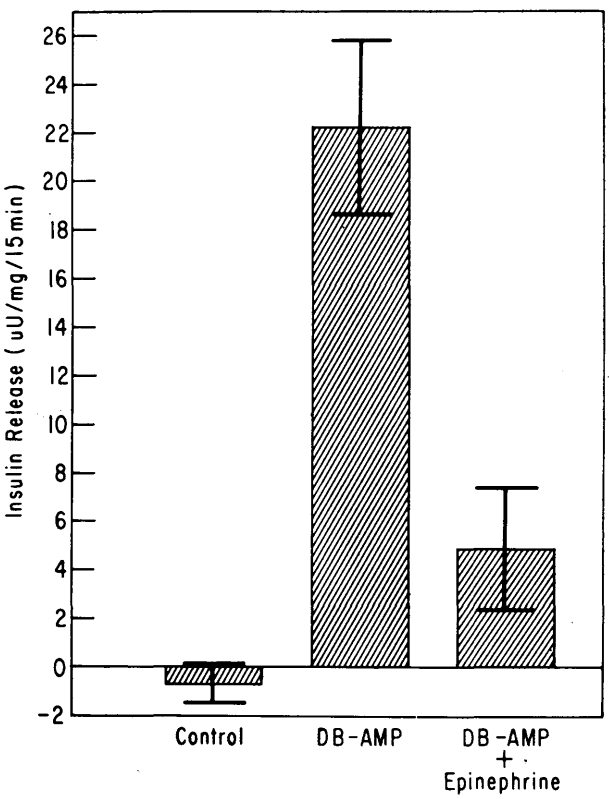

FIG. 4. Effect of epinephrine on DB-AMP mediated insulin release. DB-AMP causes a significant increase in insulin secretion as compared to the control group ($p < .01$) while epinephrine significantly inhibits this insulin release ($p < .01$). The bars represent the means and the brackets the S.E.M. of twelve experimental observations. Epinephrine was present at 2.7×10^{-6} M, and DB-AMP at 1 mg./ml.

TABLE 2

Effect of epinephrine and phentolamine on DB-AMP mediated insulin release from in vitro pancreas pieces

Additions to basal media	Insulin release (μU./mg./15 min.)
DB-AMP	19.0±3.20 (11)
DB-AMP + epinephrine	3.3±2.99 (11)
DB-AMP + phentolamine	20.6±4.06 (5)
DB-AMP + phentolamine + epinephrine	27.1±3.71 (5)

DB-AMP is present in a concentration of 1 mg./ml., epinephrine 2.7×10^{-6} M, and phentolamine 10^{-4} M. Data are expressed as mean ± S.E.M. The number in parentheses is the number of experimental observations. Epinephrine significantly inhibits DB-AMP mediated insulin release ($p < .01$). Phentolamine prevents inhibition of DB-AMP mediated insulin release by epinephrine ($p < .01$).

Epinephrine and methysergide maleate

The data therefore indicated that the mechanism of the inhibitory effects of serotonin and epinephrine on insulin release are quite similar or possibly identical. Studies were then carried out with methysergide maleate, a specific serotonin blocking agent[11] to see if this substance prevents the inhibition of insulin release by epinephrine, as it had previously been shown to do with serotonin.[12]

Figure 5, experiment 1, illustrates the effect of epinephrine on glucose-mediated insulin release. Figure 5, experiment 2, shows that methysergide maleate (Sansert) is unable to block this inhibitory effect of epinephrine on glucose-mediated insulin release. This is in contrast to experiments previously described in which methysergide maleate blocked the inhibitory effects of serotonin on insulin release.[12]

DISCUSSION

In the studies of Coore and Randle, the inhibitory effect of epinephrine on insulin secretion from in vitro rabbit pancreas was described. This epinephrine inhibition was prevented by the adrenergic blocking agent ergotamine tartrate.[1] In subsequent studies in man, epinephrine[2] and norepinephrine[3] were found to be inhibitors of insulin release, while isoproterenol was found to be a stimulator of insulin release.[5] This information, when combined with additional data obtained by using α adrenergic blocking agents such as phentolamine, and β adrenergic blocking agents such as propranolol, has given rise to the hypothesis that beta

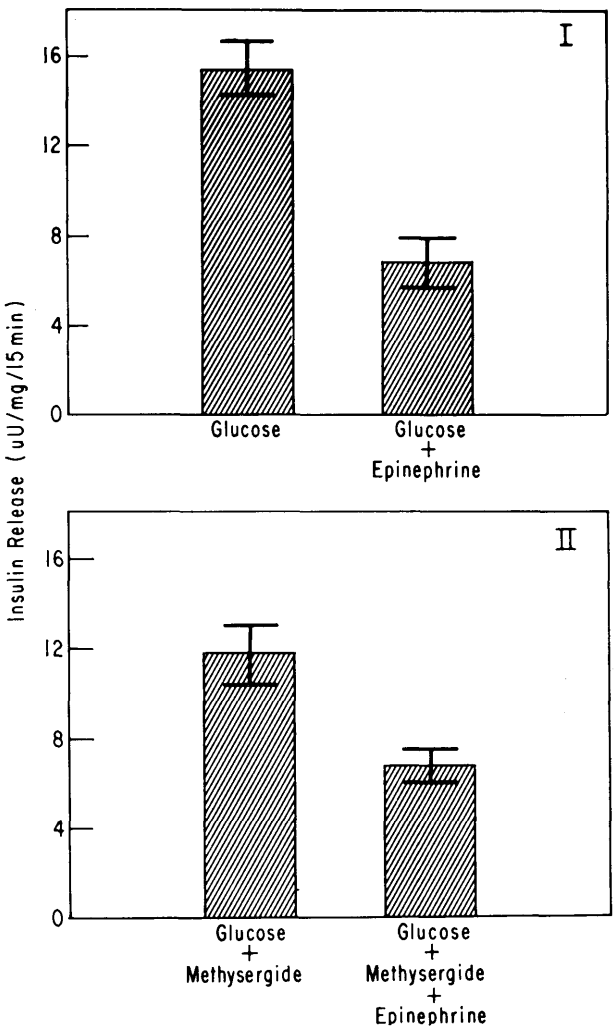

FIG. 5. *Effect of methysergide on the epinephrine inhibition* of glucose-mediated insulin release. Experiment one demonstrates the inhibition of glucose (3 mg./ml.) mediated insulin release by epinephrine ($p < .01$). Experiment two demonstrates that this epinephrine inhibition cannot be prevented by methysergide ($p < .01$). The bars represent the means and the brackets the S.E.M. of twenty-three observations per group in experiment one and fifteen observations per group in experiment two. Epinephrine was present at 2.7×10^{-6} M and methysergide was present at 10^{-4} M.

cell α receptors inhibit insulin release while beta cell β adrenergic receptors stimulate insulin release.[4,6,13]

Because epinephrine and other hormones such as glucagon and ACTH were known to both influence insulin secretion from pancreas and to affect the generation of 3′5′ cyclic AMP in nonpancreatic tissues, several groups explored the possibility that their pancreatic action was also mediated by this mononucleotide. Turtle et al. infused theophylline, a compound known to inhibit cyclic nucleotide phosphodiesterase, into adrenalectomized rats, and noted a marked rise in the serum insulin levels in the animals.[14] They attributed the elevated insulin levels to an increase in the steady state levels of 3′5′ cyclic AMP in the pancreatic beta cells. Sussman and Vaughn noted that ACTH, glucagon and cyclic AMP all resulted in an increased secretion of insulin from isolated perfused pancreas.[15]

In further studies Turtle and Kipnis directly measured the cyclic AMP levels of isolated islets after exposure to theophylline, epinephrine, phentolamine, and propranolol in varying combinations.[6] They noted that epinephrine decreased both the tissue 3′5′ cyclic AMP content, and the insulin secretion caused by theophylline. Phentolamine prevented the inhibitory effects of epinephrine on both cyclic AMP levels and insulin secretion, while propranolol did not alter the epinephrine inhibition.

The stimulatory role of the β adrenergic receptor site remains unproven. Recent studies have shown that after the administration of β adrenergic blocking agents there is marked impairment in insulin secretion in the intact mouse,[9] dog,[16] and human subjects.[16] This blocking effect has been attributed by some investigators to a specific β adrenergic blockade[10,16] and by others to a nonspecific local effect.[9] This blocking action includes the insulin release mediated by glucose,[9,10] sulfonylureas,[16] ACTH,[9] glucagon,[9] or cyclic 3′5′ AMP. In contrast, however, Allison found that β adrenergic blockage by propranolol in normal volunteers affected neither glucose tolerance nor plasma insulin secretion,[17] while Sussman et al. found that propranolol infusion actually stimulated insulin release from the perfused pancreas.[18]

In the present in vitro studies there was no direct inhibitory effect on glucose-mediated insulin secretion by propranolol despite the presence of this substance in up to 5×10^{-4} M concentration. One possible explanation for the varying results described by different investigators studying β adrenergic blockage is that the type of adrenergic receptor mediating insulin release may vary from species to species. Such species variation has previously been described for the adrenergic receptors mediating hepatic glycogenolysis.[19] A second explanation is that the effects on insulin release by β blockage may be indirect, for when an agent such as propranolol is given to the intact organism it produces many metabolic effects, and insulin secretion may be only secondarily affected. The concept of an indirect effect is supported by the recent report that

propranolol diminishes glucose-mediated insulin secretion in the intact fed rat but has no effect on glucose mediated insulin secretion in the fasted animal.[20]

It had been difficult to examine directly the thesis that epinephrine affects insulin secretion by altering the formation of cyclic AMP because most in vitro pancreas systems did not respond well to cyclic AMP. With the exception of one perfusion system,[15] they responded only in the presence of theophylline or elevated media glucose.

In the present hamster system the dibutyryl derivative was used because of its greater tissue penetrability.[21] This mononucleotide in the presence of a low glucose concentration resulted in striking increases in insulin release and allowed us to demonstrate that epinephrine and serotonin interfere with the action of cyclic AMP and not simply the generation of this nucleotide.

The prevention of the serotonin and epinephrine inhibition of DB-cyclic AMP mediated insulin release was an unexpected finding. However it is not completely without precedent, for in other systems it appears that "adrenergic blocking" drugs may not always be specific and they may also act beyond the postulated adrenergic receptor sites. Thus dehydroergotamine prevents the cyclic AMP mediated hyperglycemia in intact rats,[22] and phentolamine prevents DB-AMP mediated lipolysis.[23] From lipolysis dose response curves carried out with epididymal adipose tissue, Stock and Westerman suggested that β-adrenolytics primarily inhibit the formation of 3′5′ cyclic AMP, while α-adrenolytics inhibit the action of the cyclic nucleotide.[24]

From their studies Turtle and Kipnis concluded that epinephrine inhibits insulin release by blocking the generation of 3′5′ cyclic AMP, and phentolamine overcomes this by blocking the α receptor. The inhibitory action of epinephrine on cyclic AMP action and the prevention of this inhibitory effect by phentolamine described in the present studies suggest a quite different site of action. While our data do not explain this difference it is possible that this is due to species variation, for Turtle and Kipnis utilized the rat while we used the hamster. An alternative explanation might be that phentolamine acts at two different sites: the generation of cyclic AMP at the adrenergic receptor and the action of cyclic AMP within the cell. Additional studies will be necessary for further clarification.

The present studies emphasize the striking similarities between the action of serotonin and epinephrine.[12] The inhibitory action of these monoamines on insulin secretion could not be distinguished by either α or β adrenergic blocking agents. The only point of difference noted was that while methysergide maleate prevented the inhibitory effect of serotonin it did not alter the inhibitory effect of epinephrine. This suggests that there may be a specific serotonin receptor site in the beta cell.

The present studies do not support the thesis that insulin release due to glucose is via a beta adrenergic stimulus. They also indicate that epinephrine and serotonin inhibit insulin release by interfering with 3′5′ cyclic AMP action and phentolamine blocks this interference with 3′5′ cyclic AMP action. If this action of phentolamine is truly a manifestation of its α receptor blocking action, then at least one loci of the α receptor is in the mediation of cyclic AMP action.

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Malnutrition and Myelination

There are two mechanisms by which long chain fatty acids are synthesized: a *de novo* process leading primarily to palmitic acid, and a chain elongation process involving successive additions of acetate units to the primary fatty acid (S. J. Wakil, *J. Lipid Res. 2*:1, 1961). *In vitro* studies of brains from adult rats have demonstrated that chain elongation is the overwhelmingly prevalent mechanism for both mitochondrial and microsomal fraction (E. Aeberhard and J. H. Menkes, *J. Biol. Chem. 243*:3834, 1968). Moreover myelin, prepared by ultracentrifugation, was almost completely inactive with respect to fatty acid synthesis *in vitro*.

Fatty acid biosynthesis in subcellular particles of the developing rat brain was studied (Aeberhard, J. Grippo and Menkes, *Pediat. Res. 3*:590, 1969). Total fatty acid synthesis was maximal at fifteen to sixteen days of age. Incorporation of malonyl-CoA into saturated fatty acids followed a similar time curve. Polyunsaturated fatty acid synthesis did not change significantly from 15 days of age to maturation.

These results support the concept that fatty acid chain elongation has a close time relation with myelination. The marked increase in the rate of synthesis of saturated fatty acid by the microsomal fraction which occurs at this time suggests that this system is involved in synthesis of myelin fatty acids.

It would appear from these results that these techniques provide a method for monitoring the rate of myelination during development. Malnutrition has been shown to affect myelin deposition in developing brain (J. Dobbing and E. M. Widdowson, *Brain* 88:357, 1965; W. J. Culley and R. Lineberger, *J. Nutrition 96*: 375, 1968). Incorporation of sulfatide into myelin of developing rat brain is reduced both *in vivo* and *in vitro* by malnutrition during the first three weeks of life. Moreover, the activity of galactocerebroside sulfokinase, the enzyme responsible for this incorporation, is reduced during malnutrition (H. P. Chase, J. Dorsey, and G. M. McKhann, *Pediatrics 40*:551, 1967). In human beings there is also evidence that severe early undernutrition may affect myelin deposition in brain (M. A. Fishman, A. L. Prensky, and P. L. Dodge, *Nature 221*:552, 1969).

Thus we have evidence that myelin deposition is curtailed by malnutrition during the period of active myelination. It is conceivable that along with its effects on sulfatide incorporation, undernutrition may affect the process of chain elongation in developing rat brain and in this way reduce myelin synthesis. We must await further research to answer these questions, but the techniques are now available and some answers should be forthcoming.

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