Effects of Theophylline on Glucose Kinetics in Normal and Sympathectomized Rats

L. Sacca, M.D., G. Perez, M.D., F. Rengo, M.D., I. Pascucci, B.Sc., and M. Condorelli, M.D., Naples, Italy

SUMMARY

The influence of intraperitoneal administration of aminophylline on the rate of hepatic glucose production and peripheral uptake (Ra and Rd) was studied in normal and in adrenodemedullated and reserpinized rats by using the primed constant infusion of Glucose-2-^{3}H. In normal rats, the dose of 100 mg. per kilogram of aminophylline produced a marked increase of Ra and Rd. Since Ra rose more rapidly than Rd did initially, hyperglycemia developed. Thereafter, glucose production and uptake increased to nearly the same extent, and a new steady state was reached at plasma glucose levels almost twice those of the baseline. Smaller and transient modifications were observed after the administration of 20 mg. per kilogram of aminophylline. With the higher dose, insulin levels markedly rose (reaching a tenfold peak above the basal value) while minor increments were observed with the lower dose. In a group of normal rats which were given glucose (10 mg. per kilogram per minute) in order to achieve a degree of hyperglycemia comparable to that brought about by the higher dose of aminophylline, an almost identical enhancement of glucose uptake was recorded. However, insulin levels were much higher in aminophylline-treated rats as compared to normal rats. From these findings it was concluded that aminophylline induces resistance to insulin effect. When aminophylline was injected into demedullated rats pretreated with reserpine, at the dose of 100 mg. per kilogram, a marked enhancement of Ra, and consequently of glycemia, was recorded initially; later, severe hypoglycemia developed depending on both a progressive exhaustion of hepatic glucose production and a marked increase of glucose utilization. Insulin levels dramatically increased in these experiments. These results suggest that aminophylline directly increases glucose production by the liver and insulin secretion. The simultaneous activation of the sympathetic system blunts the insulin response and counteracts the restraining effect of insulin on the liver and the stimulatory effect of insulin on overall glucose uptake as well. DIABETES *24*:249-56, March, 1975.

The possibility that methylxanthines influence blood glucose levels and insulin secretion has long been recognized. Depending mainly on the dose employed, either an increment or no relevant modification of glycemia were observed after theophylline administration.[1-8] Furthermore, the hyperglycemic effect has been ascribed to catecholamine release induced by the methylxanthine because it disappeared after reserpinization in adrenal demedullated rats.[6] It is also firmly established that theophylline stimulates the release of insulin in vivo and in vitro.[5,9] In addition, this drug may enhance the insulin response to glucose infusion in prediabetic subjects.[10] These effects of theophylline are currently referred to as the intracellular accumulation of cyclic AMP which arises from the inhibition of phosphodiesterase activity.[11]

More recent studies have also suggested that aminophylline induces glucose intolerance in normal and hypophysectomized rats in spite of the marked enhancement of insulin response to glucose loading; the occurrence of peripheral resistance to insulin effect has been proposed as a likely explanation for this phenomenon.[8,12]

In view of these findings, we considered it of great interest to evaluate quantitatively the influence of different doses of theophylline on hepatic glucose production and peripheral uptake by tissues. The pattern of insulin secretion in response to theophylline was also examined and was related to the changes in glucose turnover. Finally, experiments were performed in sympathectomized animals in order to establish whether theophylline affects glucose metabolism through a sympathetic mediation; on the other hand, the lack of hyperglycemia which was previously found to occur in sympathectomized rats does not necessarily

From the Istituto di Patologia Speciale Medica e Metodologia Clinica, II Facoltà di Medicina, Università di Napoli, Naples, Italy.

Address reprint requests to: L. Saccà, M.D., Istituto di Patologia Medica, II Facoltà di Medicina, via Pansini 5, Napoli, Italy.

Accepted for publication November 8, 1974.

exclude the possibility of alterations in glucose dynamics.

MATERIALS AND METHODS

The experiments were conducted in normal and demedullated male rats (weighing about 300 gm.) which were maintained on commercial pellet diet. Adrenal demedullated rats were purchased from Hormone Assay Laboratories, Chicago, and were used four to five weeks after operation. After overnight fasting (eighteen to twenty hours), the animals were anesthetized with sodium thiopental (45 mg. per kilogram), and the trachea was cannulated. A heating lamp was used to ensure normal body temperature throughout the experiment.

The glucose turnover was estimated by the primed constant infusion technic according to the method of Steele, with slight modifications.[13] Extensive details of this experimental approach are reported in a previous paper.[14] Briefly, a priming dose of radioglucose (D-Glucose-2-^{3}H, Amersham, S.A. 500 mCi per mmole) was administered intravenously at zero time, followed immediately by a constant infusion which was continued throughout the experimental period. The ratio of the priming dose to the constant infusion was 40:1. Blood samples were collected through a catheter placed in the jugular vein at seventy to ninety minutes in order to calculate the baseline values of glucose turnover. At ninety minutes, aminophylline (theophylline ethylenediamine) was injected intraperitoneally into normal or sympathectomized animals; two different doses of the drug were employed—20 or 100 mg. per kilogram. Totally sympathectomized rats were prepared by adrenal medullectomy combined with reserpine, which was injected intraperitoneally twenty-four hours before the experiments at the dose of 2.5 mg. per kilogram.* The effectiveness of this treatment was confirmed by the lack of a hyperglycemic response to coldness (4° C.).

In order to better evaluate the effects of aminophylline on glucose uptake by tissues, in another group of experiments glucose kinetics and insulin secretion were measured in normal rats receiving glucose by constant intravenous infusion at the rate of 10 mg. per kilogram per minute. This dose of glucose produced hyperglycemia of comparable degree to that brought about by the high dose of aminophylline. In two other experiments glucose was infused at the rate of 20 or 30 mg. per kilogram per minute together with pork insulin (2 or 4 mU. per minute).

In order to determine the specific activity of plasma glucose, 50 μL. of plasma for each sample were deproteinized with $Ba(OH)_2$-$ZnSO_4$. An aliquot of the supernatant was used for glucose assay by the glucose oxidase method (Boehringer, Mannheim GmbH). Another aliquot was evaporated to dryness under vacuum at 70° C. to remove the ^3H_2O content of the plasma. This simple procedure permits complete isolation of Glucose-2-^{3}H, since most of the tritium is irreversibly lost as water from position 2 in the isomerization of the hexose-6-phosphates.[15,16] The dry residue was dissolved in 1 ml. of water and mixed with 9 ml. of Insta-Gel (Packard Instrument). Glucose specific activity was then expressed as m μCi per milligram. Insulin radioimmunoassay was performed by the method of Herbert et al. using rat insulin as standard.[17]

In the basal state, since negligible fluctuations in plasma glucose concentrations and specific activity occurred, the glucose turnover rate (milligrams per kilogram body weight per minute) was calculated by the general equation applicable to all steady state systems: Rt = Ra = Rd = r/SA, where Rt = the rate of glucose turnover, Ra = the rate of hepatic glucose production, Rd = the rate of overall glucose uptake, r = the rate of infusion of the tracer (m μCi per kilogram per minute), and SA = the specific activity of glucose at equilibrium (m μCi per milligram). In nonsteady states it is possible to calculate with acceptable approximation Ra and Rd using Steele's equations (equations 4a and 5a)[13] which give the averaged values of the rates of glucose turnover for the interval between two consecutive samplings. The metabolic clearance rate of glucose (MCR) (milliliters per kilogram per minute) was calculated as the ratio Rd/plasma glucose (milligrams per milliliter).

All data are presented as mean ± S.E. Statistical significance of the difference among two means was estimated by the Student t test. When the variances of the groups to be compared were unequal, the Cochran test was employed.[18]

RESULTS

Figure 1 depicts the metabolic changes induced by the administration of the high dose of aminophylline (100 mg. per kilogram) to normal rats. Plasma glucose progressively increased during the first hour and

*Although the operation is a sympathectomy in a functional sense only, the animals will be indicated as sympathectomized rats.

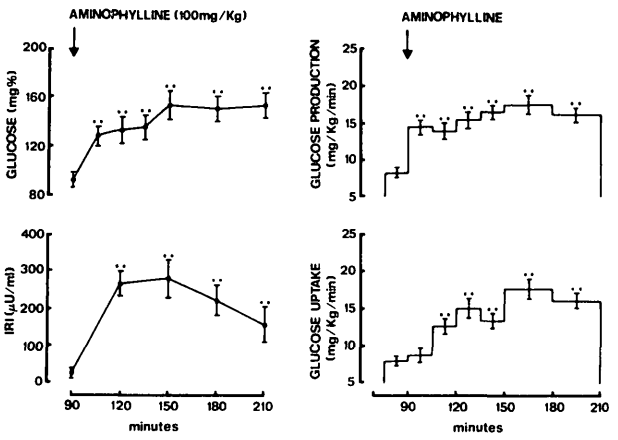

FIG. 1. Effect of the administration of aminophylline (100 mg. per kilogram) on plasma concentrations of glucose and insulin and on the rates of hepatic glucose production and overall uptake by tissues in normal rats. One or two asterisks indicate $p<0.05$ and $p<0.01$, respectively. (number of rats = 8)

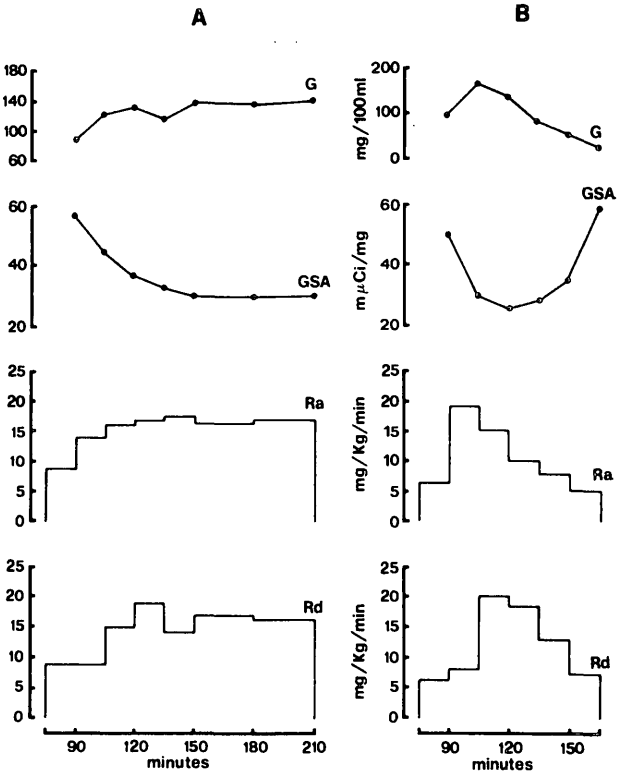

FIG. 2. A. Effect of the administration of 100 mg. per kilogram of aminophylline on plasma glucose (G), glucose specific activity (GSA), hepatic glucose production (Ra), and over-all glucose uptake (Rd) in a normal rat weighing 252 gm. At zero time, a priming dose of Glucose-2-^{3}H was given intravenously followed by a constant infusion of tracer at the rate of 128 m μCi per minute. B. Effect of the administration of 100 mg. per kilogram of aminophylline to a sympathectomized rat weighing 325 gm. G, GSA, Ra, Rd as in A. The rate of the tracer infusion was 103 m μCi per minute.

then stabilized (at a plateau) during the second hour of the experiments. Hyperglycemia was supported by a more pronounced enhancement of hepatic glucose production than glucose uptake in the early phase of the experiments. Thereafter, the pattern of Ra and Rd change was similar and, therefore, a new steady state was reached. Furthermore, a spectacular increase of insulin secretion with a peak of tenfold the basal value was observed at sixty minutes after the administration of aminophylline. No important modification of the metabolic clearance of glucose occurred (table 1). One representative experiment of this group is shown in figure 2.

The effects induced by the low dose of aminophylline (20 mg. per kilogram) were transient and of minor extent as compared to the high dose (figure 3). In fact, a moderate but significant hyperglycemia and a simultaneous increment of glucose production occurred only at fifteen minutes; these modifications were accompanied by a short-lived enhancement of glucose uptake. Insulin levels also moderately increased thirty and sixty minutes after aminophylline and then slowly declined.

In figure 4 are illustrated the metabolic changes induced by the infusion of glucose at the rate of 10 mg. per kilogram per minute in a group of normal rats. The plasma glucose levels and the rate of overall glucose uptake were essentially identical to those ob-

TABLE 1

Metabolic clearance rate (milliliters per kilogram per minute) of glucose in normal rats loaded with glucose at the rate of 10 mg. per kilogram per minute, in normal rats treated with 100 mg. per kilogram of aminophylline, and in sympathectomized rats treated with the same dose of aminophylline

minutes	0	0-15	15-30	30-45	45-60	60-90	90-120
Glucose infusion (12)*	10.2±0.3	9.9±0.4	10.6±0.3	11.4±0.3	10.3±0.4	11.0±0.5	10.7±0.4
Aminophylline in normal rats (8)	8.8±0.5	7.7±0.9	10.1±1	11.5±1.3	9.6±1.1	11.8±1.2	10.8±0.8
Aminophylline after sympathectomy (6)	8.4±0.6	9.4±1.6	16.6±2.3	19.5±2.2	14.8±2.1		

*Number of rats in parentheses

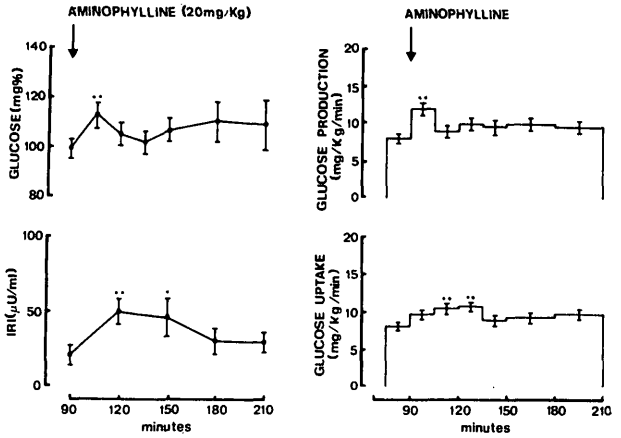

FIG. 3. Effect of the administration of aminophylline (20 mg. per kilogram) on plasma concentrations of glucose and insulin and on the rates of hepatic glucose production and overall uptake by tissues in normal rats. One or two asterisks indicate $p<0.05$ and $p<0.01$, respectively. (number of rats = 10)

served in normal rats treated with 100 mg. per kilogram of aminophylline, while insulin levels were much higher in aminophylline-treated rats at all time intervals ($p<0.005$ - $p<0.001$). Incidentally, it must be noted that in these experiments glucose infusion only moderately reduced the rate of appearance of glucose and did not give rise to appreciable increase of the metabolic clearance rate (table 1). This unexpected behavior, which closely resembles that described recently in newborn dogs,[19] could be explained in terms of low sensitivity of the glucoregulatory system in the rat. In fact, when exogenous glucose was infused together with a large amount of insulin, glucose output was almost completely suppressed, and the metabolic clearance of glucose gradually rose to a level that was more than twice the preinfusion value (figures 5 and 6). Interestingly, in the experiment depicted in figure 6, plasma glucose and insulin stabilized at levels very near to those observed in rats treated with the higher dose of aminophylline while glucose uptake was much higher and was accompanied by a parallel increase of the metabolic clearance rate.

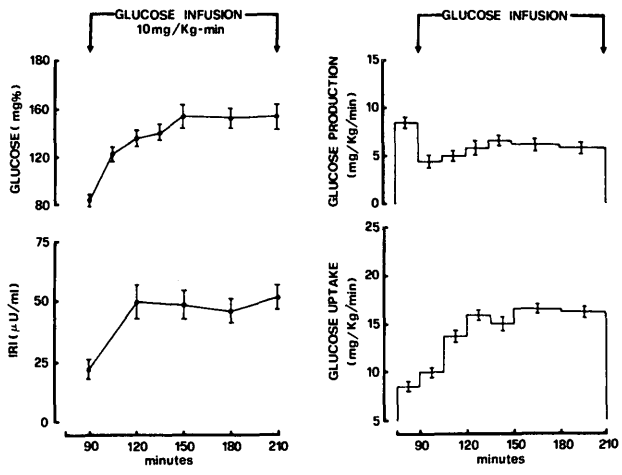

FIG. 4. Effect of the intravenous infusion of glucose at the rate of 10 mg. per kilogram per minute on plasma concentrations of glucose and insulin and on the rates of hepatic glucose production and overall uptake by tissues in twelve normal rats.

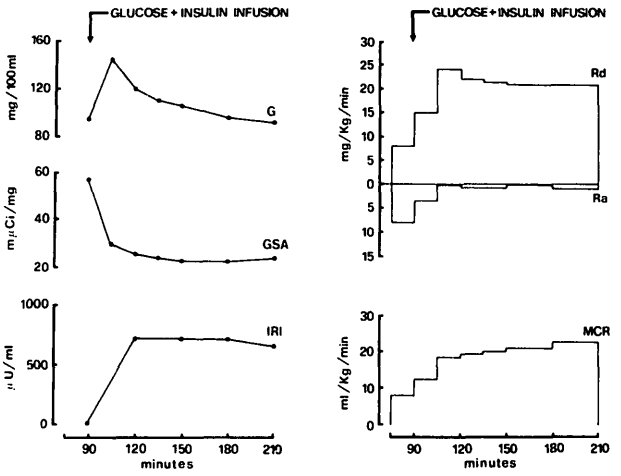

FIG. 5. Effect of intravenous infusion of glucose plus insulin (20 mg. per kilogram per minute plus 4 mU. per minute) on plasma glucose (G), glucose specific activity (GSA), insulin (IRI), rate of glucose production (Ra), rate of overall glucose uptake (Rd), and metabolic clearance rate of glucose (MCR) in a normal rat weighing 320 gm. The rate of the tracer infusion was 154 m μCi per minute.

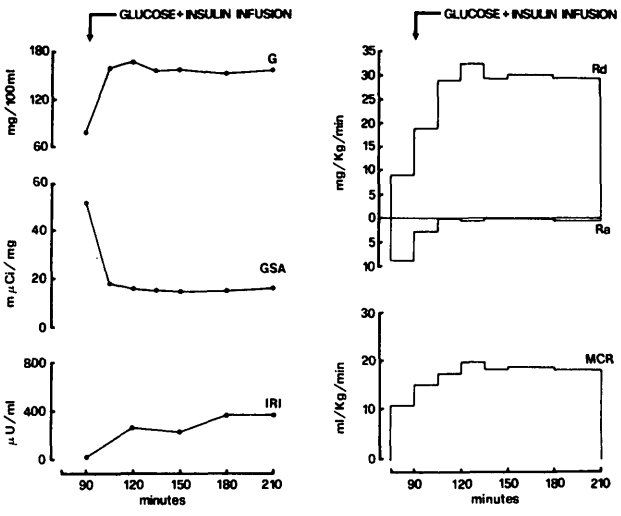

FIG. 6. Metabolic changes induced by the infusion of glucose plus insulin (30 mg. per kilogram per minute plus 2 mU. per minute) in a normal rat weighing 335 gm. The rate of the tracer infusion was 160 m μCi per minute. Symbols used are the same as in figure 5.

In the sympathectomized rats treated with the high dose of aminophylline, plasma glucose, after an initial increment, showed a progressive decline until lethal hypoglycemia developed. Because of the different survival time, the results of these experiments are reported singly in table 2. The occurrence of severe hypoglycemia in these animals depends on two factors. First, the progressive and marked decrease in the rate of glucose production which followed the transient increase immediately after the administration of aminophylline. Second, the strong enhancement of glucose uptake by tissues which was not counterbalanced by a parallel increase in hepatic production. The metabolic clearance rate of glucose significantly increased, achieving a level that was more than twice the basal value (table 1). Plasma insulin concentrations dramatically increased in all the experiments and in some cases exceeded the value of 1 mU. per milliliter.

In the sympathectomized animals given the low dose of aminophylline, plasma glucose, after an initial enhancement, declined slowly without reaching critical values, however. The rates of glucose turnover increased in the first times following aminophylline administration and then stabilized at levels near the basal value. With respect to insulin levels, a great variability of the results was observed. However, a significant increase of about sixfold the basal value was recorded thirty minutes after aminophylline administration (table 3).

DISCUSSION

Although the hyperglycemic action of theophylline has long been recognized, no attempt has been made as yet to quantitate it in kinetic terms. Our studies demonstrate that the hyperglycemia which follows the administration of a high dose of aminophylline (100 mg. per kilogram) to normal rats is supported by an enhanced rate of appearance of glucose which under these experimental conditions is to be assumed to represent the glucose production by the liver. The overall uptake of glucose increased more slowly than glucose production in the initial phase; thereafter, both rates stabilized at the same high levels and a new steady state was achieved. Conversely, the low dose of aminophylline (20 mg. per kilogram) did not produce gross modifications of the parameters under study, except for a slight increase in the initial phase. The lack of hyperglycemic response to low doses of theophylline (<75 mg. per kilogram) has been reported also by other investigators.[1-3] Therefore, it may be concluded from our studies that normo-

TABLE 2

Changes in plasma glucose (G), hepatic glucose production (Ra), overall glucose uptake (Rd), and insulin levels (IRI) induced by the administration of 100 mg. per kilogram of aminophylline in sympathectomized rats

		minutes after aminophylline administration					
Exp. no.		0	0-15	15-30	30-45	45-60	60-75
22 AM	G	109	163	194	141	72	27
	Ra	8.6	18.8	19.8	21.2	5.9	1.5
	Rd	8.6	9.8	14.6	30.0	17.3	5.3
	IRI	18		700		156	
23 AM	G	98	164	133	82	51	28
	Ra	6.3	19.1	15.0	10.1	7.7	5.0
	Rd	6.3	8.1	20.2	18.6	12.9	6.9
	IRI	22		368		48	
24 AM	G	108	211	161	53	31	
	Ra	8.6	31.4	14.7	2.1	3.5	
	Rd	8.6	14.4	30.5	12.6	7.2	
	IRI	30		460		160	
30 AM	G	95	142	100	42	23	
	Ra	9.7	17.5	15.0	5.9	0	
	Rd	9.7	9.5	22.1	15.6	2.7	
	IRI	19		1,040		240	
53 AM	G	98	135	82	50	65	36
	Ra	9.7	17.5	13.4	9.5	9.9	2.7
	Rd	9.7	11.3	22.1	14.9	7.4	5.0
	IRI	18		368		48	
61 AM	G	100	113	43	26		
	Ra	8.0	19.4	5.8	0		
	Rd	8.0	17.2	17.7	2.8		
	IRI	20		1,200			

TABLE 3

Changes in plasma glucose, hepatic glucose production, overall glucose uptake, and insulin levels induced by the administration of 20 mg. per kilogram of aminophylline in sympathectomized rats (number of rats = 6)

minutes	0	0-15	15-30	30-45	45-60	60-90	90-120
Plasma glucose (mg./100 ml.)	91 ±5	117 ±8*	88 ±4	75 ±6	75 ±6	72 ±8	64 ±12
Glucose production (mg./kg/min.)	8.2±0.6	13.8±1.6*	9.0±0.7	8.8±0.8	9.0±0.6	8.7±1.0	7.6± 1.6
Glucose uptake (mg./kg./min.)	8.2±0.6	9.4±0.8	13.8±1.6*	11.2±0.4†	9.0±0.8	9.0±0.8	8.2± 1.5
Insulin (μU./ml.)	23 ±4		123 ±38*		41 ±10	52 ±14	80 ±29

*$p<0.05$
†$p<0.01$

glycemia which occurs using low doses of theophylline does really reflect the incapacity of methylxanthine to modify the rate of glucose turnover.

As far as insulin secretion is concerned, a significant increase of plasma insulin levels was observed with both doses of aminophylline. However, such response was much more marked with the high dose which produced a tenfold increment. Surprisingly, this increment of plasma insulin was paralleled by a simultaneous enhancement of plasma glucose which stabilized at high levels in spite of the sustained hyperinsulinism. In the light of these results, it was conceivable to hypothesize that insulin resistance might be engendered by aminophylline, as previous investigators suggested on the basis of the impairment of the glucose tolerance test in rats treated with aminophylline.[8,12] In the present study we tried to further elucidate this aspect using a kinetic model and relating the glucose utilization by tissues to the plasma concentrations of insulin. In this regard, the experiments summarized in figure 4 suggest that aminophylline produces resistance of the tissues to insulin effect. In fact, an almost identical enhancement of glucose uptake required fivefold higher insulin levels in aminophylline-treated rats in comparison to glucose-loaded animals. However, it could be thought that the higher insulin levels in aminophylline-treated rats failed to further increase the rate of disappearance of glucose since a maximal value of Rd had been reached already. In this regard, it must be noted that, in rats loaded with glucose along with a large amount of exogenous insulin, Rd and MCR achieved much higher values than those observed in normal rats receiving aminophylline. In particular, the experiment depicted in figure 6 lends further support to the concept of insulin resistance being engendered by aminophylline since plasma glucose and insulin levels were very near to those recorded in rats injected with the high dose of aminophylline, while glucose uptake and MCR displayed a more consistent increase.

It is well known that, in the rat, theophylline increases plasma FFA[2-4] which can affect glucose utilization by tissues. Because of the large volume of plasma required, FFA were not assayed in our experiments. However, very likely they do not play a causal role in the development of insulin resistance since it has been reported that aminophylline administration causes glucose intolerance also in the absence of an increase of FFA levels.[8]

Many studies indicate that methylxanthines enhance adrenal medullary release and urinary excretion of catecholamines.[20-23] For this reason we have performed some experiments in sympathectomized rats in order to further elucidate the mechanism of aminophylline interaction with glucose metabolism. Although catecholamines were not assayed in these experiments, our findings prompt us to speculate that aminophylline per se is able to accelerate glucose turnover regardless of adrenergic mediation. They also suggest that sympathetic basal tone is not a prerequisite for the stimulatory action of aminophylline on hepatic glucose metabolism. In other words, the inhibition of phosphodiesterase induced by aminophylline can lead to cyclic AMP accumulation in an amount sufficient to activate the release of glucose by the liver even in the absence of a basal sympathetic tone. The extreme reduction of glucose production which occurs after the initial increment can be attributed to the simultaneous spectacular enhancement of insulin levels. Insulin, in fact, lowers the liver content of cyclic AMP and thereby suppresses glucose production.[24] On the other hand, the finalistic glucose-mobilizing effect of hypoglycemia cannot take

place in these conditions presumably for two reasons. The first is that in the rat, in contrast to other animal species, the "restraining effect" of insulin largely predominates over the glucose-mobilizing effect of hypoglycemia as recent evidence indicates.[25] The second is the lack of sympathetic function. In fact, the administration of aminophylline to normal rats results in a remarkable increase of hepatic glucose production and plasma glucose level despite the concomitant high levels of circulating insulin whose "restraining effect" is overriden by the combined action of catecholamines and aminophylline. By contrast, comparable insulin levels, obtained by the infusion of glucose and insulin to normal rats in whom no sympathetic overactivity is acting, do suppress almost completely glucose production by the liver (figure 6). A similar situation very likely occurs in sympathectomized rats in whom the release of catecholamines cannot be evoked by aminophylline and, therefore, the lowering action of insulin on hepatic cyclic AMP is no longer counteracted by the stimulatory effect of catecholamines.

The very marked insulin response observed in sympathectomized animals can be explained by the insulin-releasing effect of aminophylline not being blunted by catecholamines which are well known inhibitors of insulin release.[26,27] In addition, catecholamines have been reported to inhibit glucose uptake by tissues in "in vivo" tracer studies[28] and by rat diaphragm "in vitro".[29,30] On the other hand, an inhibiting effect on glucose utilization is also exerted by cyclic AMP "in vivo"[31] and by cyclic AMP and theophylline on rat diaphragm "in vitro" in the presence of insulin.[32,33] In line with these observations, the more marked increase of Rd and MCR in sympathectomized rats as compared to normal rats receiving the same dose of aminophylline could be theoretically ascribed both to the lack of endogenous catecholamines and to the more pronounced insulin response. However, we are led to speculate that insulin exerts a more marked effect on Rd and MCR because of the lack of catecholamines since these parameters consistently increased in all the experiments even when insulin levels were not higher but rather of the same magnitude as those observed in normal rats receiving aminophylline.

The following general conclusion can be drawn from our results: in intact rats, aminophylline stimulates insulin release and enhances glucose turnover. The simultaneous activation of the sympathetic system prevents the disruption of the metabolic homeostasis by limiting the insulin response and counteracting the "restraining effect" of insulin on the liver and the stimulating effect of insulin on overall glucose uptake as well. In the sympathectomized rat these compensatory reactions cannot take place, and lethal hypoglycemia develops.

REFERENCES

[1]Northrop, G., and Parks, R.E., Jr.: The effects of adrenergic blocking agents and theophylline on 3', 5'-AMP induced hyperglycemia. J. Pharmacol. Exp. Ther. *145*:87-91, 1964.

[2]Hynie, S., Krishna, G., and Brodie, B.B.: Theophylline as a tool in studies of the role of cyclic adenosine 3', 5'-monophosphate in hormone-induced lipolysis. J. Pharmacol. Exp. Ther. *153*:90-96, 1966.

[3]Triner, L., and Nahas, G.G.: Effects of theophylline and catecholamines on lipolysis and glycogenolysis in vivo. J. Pharmacol. Exp. Ther. *153*:569-72, 1966.

[4]Poyart, C., and Nahas, G.G.: Metabolic effects of theophylline and norepinephrine in the dog at normal and acid pH. Am. J. Physiol. *212*:1247-54, 1967.

[5]Turtle, J.R., Littleton, G.K., and Kipnis, D.M.: Stimulation of insulin secretion by theophylline. Nature (Lond) *213*:727-28, 1967.

[6]Strubelt, O.: The influence of reserpine, propranolol, and adrenal medullectomy on the hyperglycemic action of theophylline and caffeine. Arch. Int. Pharmacodyn. Ther. *179*:215-24, 1969.

[7]Ensinck, J.W., Stoll, R.W., Gale, C.C., Sauten, R.J., Touber, J.L., and Williams, R.H.: Effect of aminophylline on the secretion of insulin, glucagon, luteinizing hormone and growth hormone in humans. J. Clin. Endocrinol. Metab. *31*:153-61, 1970.

[8]Penhos, J.C., Castillo, L., Ezekiel, M., Yamaguchi, N., De Santis, R.A., Gutman, R.A., and Recant, L.: Insulin-resistant hyperglycemia produced by aminophylline. Endocrinology *90*:131-37, 1972.

[9]Malaisse, W., Malaisse-Lagae, F., and Mayhew, D.: A possible role for the adenylcyclase system in insulin secretion. J. Clin. Invest. *46*:1724-34, 1967.

[10]Cerasi, E., and Luft, R.: The effect of an adenosine-3', 5'-monophosphate diesterase inhibitor (aminophylline) on the insulin response to glucose infusion in prediabetic and diabetic subjects. Horm. Metab. Res. *1*:162-68, 1969.

[11]Butcher, R.W., and Sutherland, E.W.: Adenosine 3', 5'-phosphate in biological materials: I-Purification and properties of cyclic 3', 5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3', 5'-phosphate in human urine. J. Biol. Chem. *237*:1244-49, 1962.

[12]De Santis, R.A., Garcia, M.G., Czerwinski, C., Van Lau, Vo, Gutman, R.A., Recant, L., and Penhos, J.C.: Effects of glucocorticoid and aminophylline on glucose intolerance of adrenalectomized rats. Endocrinology *92*:716-21, 1973.

[13]Steele, R.: Influence of glucose loading and of injected insulin on hepatic glucose output. Ann. N.Y. Acad. Sci. *82*:420-30, 1959.

[14]Saccà, L., Perez, G., Rengo, R., and Condorelli, M.: Effects of different prostaglandins on glucose kinetics in the rat. Diabetes *23*:532-35, 1974.

[15]Katz, J., and Dunn, A.: Glucose-2-t as a tracer for glucose metabolism. Biochemistry 6:1-5, 1967.

[16]Hetenyi, G., Jr., and Mak, D.: ^{3}H-2-Glucose as tracer in turnover studies. Can. J. Physiol. Pharmacol. *48*:732-33, 1970.

[17]Herbert, V., Lau, K., Gottlieb, C.W., and Bleicher, S.J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. *25*:1375-84, 1965.

[18]Snedecor, G.W., and Cochran, W.G.: Statistical Methods. Iowa State University Press, 1967.

[19]Varma, S., Nickerson, H., Cowan, J.S., and Hetenyi, G., Jr.: Homeostatic responses to glucose loading in newborn and young dogs. Metabolism *22*:1367-75, 1973.

[20]De Schaepdryver, A.F.: Physio-pharmacological effects on suprarenal secretion of adrenaline and noradrenaline in dogs. Arch. Int. Pharmacodyn. Ther. *19*:517-18, 1959.

[21]Atuk, N.O., Blaydes, M.C., Westervelt, F.B., and Wood, J.E.: Effect of aminophylline on urinary excretion of epinephrine and norepinephrine in man. Circulation *35*:745-53, 1967.

[22]Levi, L.: The effect of coffee on the function of the sympatho-adrenomedullary system in man. Acta Med. Scand. *181*:431-38, 1967.

[23]Bellet, S., Roman, L., DeCastro, O., Eun Kim, K., and Kershbaum, A.: Effect of coffee ingestion on the catecholamine release. Metabolism *18*:288-91, 1969.

[24]Park, C.R., Lewis, S.B., and Exton, J.H.: Relationship of some hepatic actions of insulin to intracellular level of cyclic adenylate. Diabetes *21*:439-46, 1972.

[25]Cole, J., Achou, C.J., and Hetenyi, G., Jr.: Lack of an increase in endogenous glucose production during insulin-induced hypoglycemia in the rat. Endocrinology *93*:1438-41, 1973.

[26]Malaisse, W., Malaisse-Lagae, F., Wright, P.H., and Ashmore, J.: Effects of adrenergic and cholinergic agents upon insulin secretion "in vitro". Endocrinology *80*:975-78, 1967.

[27]Porte, D., Jr., Graber, A.L., Kuzuya, T., and Williams, R.H.: The effect of epinephrine on immunoreactive insulin levels in man. J. Clin. Invest. *45*:228-36, 1966.

[28]Altszuler, N., Steele, R., Rathgeb, I., and De Bodo, R.C.: Glucose metabolism and plasma insulin levels during epinephrine infusion in the dog. Am. J. Physiol. *212*:677-82, 1967.

[29]Waalas, O., and Waalas, E.: Effect of epinephrine on rat diaphragm. J. Biol. Chem. *187*:769-76, 1950.

[30]Kipnis, D.M., and Cori, C.F.: Studies of tissues permeability. V. The penetration and phosphorylation of 2-deoxyglucose in the rat diaphragm. J. Biol. Chem. *234*:171-77, 1959.

[31]Altszuler, N., Rathgeb, I., and Steele, R.: Mechanism responsible for the hyperglycemia produced by the dibutyryl derivative of cyclic 3′, 5′-adenosine monophosphate. Biochem. Pharmacol. *20*:2813-19, 1971.

[32]Chambaut, A.M., Eboué-Bonis, D., Hanoune, J., and Clauser, H.: Antagonistic action between dibutyryl adenosine-3′, 5′-cyclic monophosphate and insulin on the metabolism of the surviving rat diaphragm. Biochem. Biophys. Res. Commun. *34*:283-90, 1969.

[33]Ensinck, J.W., Touber, J.L., and Stoll, R.H.: Antagonism of insulin action on muscle by cyclic 3′, 5′ AMP. Diabetes *18*:337-38, 1969.