Opposite Effects of Aminophylline on Arginine-induced Glucagon and Insulin Secretion in Humans

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SUMMARY

The adenyl cyclase-cyclic AMP system, aside from being a mediator of the actions of several hormones, is believed to play an important role in the secretory mechanism of some of these hormones. In young, healthy, nonobese volunteers, we have studied the effect of a large dose (1 gm.) of a cAMP phosphodiesterase inhibitor (aminophylline) on basal plasma glucagon and insulin levels and on the responses of these two hormones to two different doses of arginine.

With a high (468 mg./kg.) and a low (156 mg./kg.) dose of arginine, similar maximal peaks of glucagon concentration were reached, indicating that the level of aminoacidemia reached with the low dose was sufficient to elicit an almost maximal acute response. Plasma insulin and glucose increased as expected after the arginine doses.

With aminophylline alone, plasma insulin and glucose increased slightly but consistently while the level of circulating glucagon did not change (range of the means, 111 pg./ml. ± 28 to 133 pg./ml. ± 25). Aminophylline pretreatment significantly lowered the glucagon response in the late part of the curve corresponding to the high dose of arginine. This inhibitory effect of the xanthine derivative could be due to: (1) peripheral effects of aminophylline, such as increased mobilization of FFA; (2) enhanced removal of glucagon from plasma; (3) direct suppressor effect of cAMP on the alpha cell secretory mechanism. At any rate, this effect of aminophylline seems to be either a retarded one or only present in the late phase of glucagon secretion. DIABETES *21*:289-94, May, 1972.

There exists evidence indicating that the nucleotide, adenosine 3′, 5′-monophosphate (cyclic AMP, cAMP), aside from being a mediator of the action of several hormones at the cellular level of the target organ, is also involved in the secretory mechanism of some of these hormones.[1]

Concerning the endocrine pancreas, it has been suggested that an increase in the concentration of cAMP inside the beta cell would be associated with an increase in the secretion of insulin.[2,3] In vitro experiments to determine the effect of cAMP on glucagon release have yielded conflicting results. Chesney and Schofield[4] have reported a stimulatory effect of theophylline on glucagon release in isolated mouse pancreatic islets incubated with 3.3 mM glucose, and they concluded that cAMP may play a role in the regulation of glucagon secretion. On the other hand, Vance and associates[5] were not able to detect any significant change in the secretion of glucagon by isolated pancreatic islets of rats when either aminophylline or dibutyryl cyclic AMP were added to an incubation medium containing 8.3 mM glucose. Ensinck et al.[6] reported that the infusion of 500 mg. of aminophylline in normal and adrenalectomized women was ineffective in altering the levels of circulating glucagon. However, these investigators point out that the antiserum used in their glucagon determinations presumably cross-reacts with gut glucagon-like immunoreactivity (GLI) and this could be the reason for the inability to detect small changes in the plasma concentration of pancreatic glucagon.

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The present work was designed in order to study the effect of a large dose of a cAMP phosphodiesterase inhibitor (aminophylline) on the basal plasma glucagon and insulin levels, and on the secretory response of the alpha and beta cells to two different doses of arginine in normal subjects.

MATERIALS AND METHODS

Young, healthy, nonobese volunteers with no clinical history of diabetes in their families were selected for our study. All of them were physicians or medical students and had been informed of the purpose and nature of the experiments. Each individual served as his own control, that is to say, when a joint aminophylline-arginine test was performed on a given subject, that subject had previously undergone an arginine test.

Volunteers reported to the laboratory between 9:00 a.m. and 10:00 a.m. after an overnight fast. Tests were carried out with the subjects in a recumbent position. For taking samples, an indwelling butterfly needle connected to a stopcock was placed in an antecubital vein and kept patent with a 0.1 per cent heparin solution. In the other arm, a similar set-up was applied for the administration of the test doses. A period of thirty minutes was allowed for relaxation before collection of the first control sample.

Aminophylline (Elmufilina, Elmu) was administered intravenously over a ten-minute period, as a solution of 1 gm. in 80 ml. of normal saline. Arginine monohydrochloride (Arginina, Hermes) was administered as a 20 per cent solution, intravenously, over a ten-minute period. Two different test doses were employed: high, 468; and low, 156 mg. per kilogram of body weight. In the experiments studying the combined effects of aminophylline and arginine, arginine administration was started immediately upon termination of the aminophylline dose.

Blood samples (10 ml.) were drawn at ten-minute intervals throughout the test. For glucose and insulin measurements, 5 ml. of blood were placed in chilled tubes containing EDTA; another 5 ml. for glucagon analysis were collected in chilled tubes containing EDTA and 2,500 U. of kallikrein-trypsin inhibitor (Trasylol, Bayer) in a volume of 0.5 ml. The blood was promptly centrifuged at 4° C., and the plasma was stored at —20° C. until the time of assay. Assays were performed within four weeks of the date of experiments.

All samples were tested in duplicate. Plasma glucose was determined by means of a commercial glucose-oxidase preparation (Biochemica Test Combination, Boehringer Mannheim GMBH). Insulin was measured by radioimmunoassay using the Herbert charcoal separation method.[7] Insulin-I^{125} was purchased from The Radiochemical Centre, Amersham, Bucks. (England). Pork insulin (Novo, Lot #S4769) was used as the standard. The antiserum (GP-14) against pork insulin was employed at a final dilution of 1:25,000. Glucagon was assayed radioimmunologically[8] with an antiserum (30 K) highly specific for pancreatic glucagon (kindly supplied by Dr. Roger H. Unger) at a final dilution of 1:60,000. Beef-pork glucagon (Eli Lilly, Lot #781416) was used as the standard. Glucagon-I^{125} was obtained from Cambridge Nuclear Corporation, Billerica, Mass. (U.S.A.).

Statistical processing of the data was performed by conventional methods.

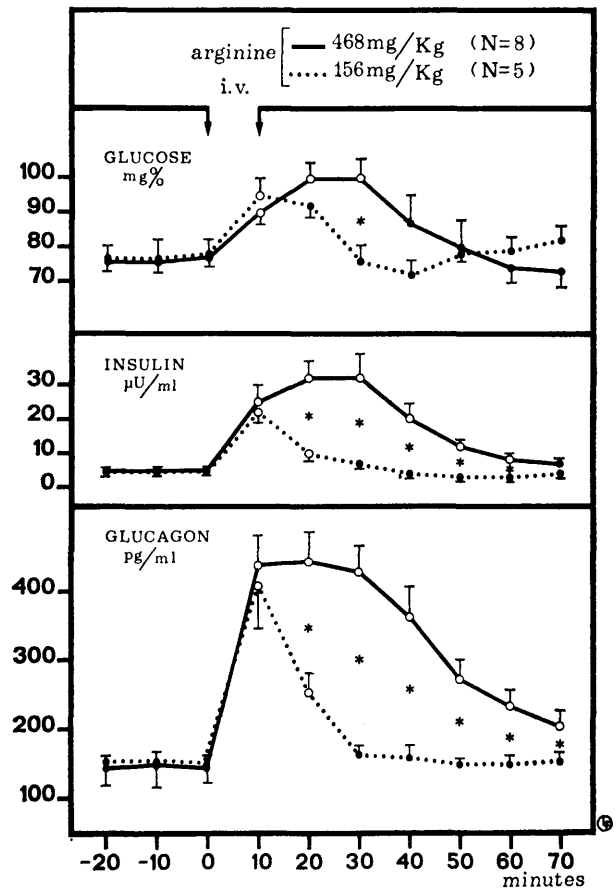

FIG. 1. Comparison of the effects of two different doses of arginine on glucagon and insulin secretion in normal subjects. The open circles represent statistically significant differences from the baseline. The asterisks represent statistically significant differences between both groups for a given time. (Mean ± S.E.M.).

RESULTS

1. *Comparison of the effects of a high (468 mg./kg.) and a low (156 mg./kg.) dose of arginine on glucagon and insulin secretion* (figure 1).

The high arginine dose elicited a marked increase in plasma glucagon concentration, from a baseline of 149 pg./ml. (S.E.M. ± 25) to 443 pg./ml. (± 44) immediately after the injection ($P < 0.001$). A plateau was maintained for the next twenty minutes and the values remained significantly elevated for the duration of the test period ($P < 0.001$ at 20 and 30 min.; $P < 0.005$ at 40 and 50 min.; $P < 0.05$ at 60 and 70 min.). With the low dose of arginine, a spike curve of glucagon values was obtained. The peak, 411 pg./ml. (± 61) ($P < 0.01$) was almost the same as that reached with the high dose. At twenty minutes, the level was still elevated ($P < 0.01$) but after thirty minutes the values returned to basal levels. The curve of glucagon values after the high arginine dose was above that for the low dose throughout the experiment. The differences were statistically significant at all points except the peak and the final sample ($P < 0.01$ at 20 min.; $P < 0.001$ at 30 min.; $P < 0.005$ at 40 min.; $P < 0.01$ at 50 min.; $P < 0.05$ at 60 min.).

The insulin response followed a similar pattern to that of glucagon. With the high arginine dose, plasma insulin concentrations were significantly elevated throughout the test with exception of the last sample ($P < 0.01$ at 10, 20, 30, 40, and 50 min.; $P < 0.05$ at 60 min.). With the low dose, the elevation was significant only at ten and twenty minutes ($P < 0.01$). The insulin response for the high dose was significantly greater than that for the low dose ($P < 0.05$ at 20 and 30 min.; $P < 0.01$ at 40 and 50 min.; $P < 0.05$ at 60 min.).

With the high arginine load, plasma glucose increased from a basal value of 77 mg./100 ml. (± 3) to 100 mg./100 ml. (± 4) at twenty minutes. The elevation was statistically significant at 10, 20, and 30 min. ($P < 0.001$). The low arginine load induced a less intense rise in plasma glucose; from 78 mg./100 ml. (± 4) to 95 mg./100 ml. (± 5) at ten minutes. Only this point was significantly elevated over the baseline ($P < 0.001$). The thirty-minute sample was significantly lower than that corresponding to the high arginine dose ($P < 0.001$).

2. *Effect of aminophylline on basal glucagon and insulin levels* (figure 2).

The intravenous administration of 1 gm. of aminophylline failed to modify the levels of circulating glucagon. The concentration of glucagon in plasma ranged from 111 pg./ml. (± 28) to 133 pg./ml. (± 25) during the entire experimental period.

Plasma insulin increased slightly but consistently and remained significantly elevated in all the post-injection points ($P < 0.01$ at 10 and 20 min.; $P < 0.001$ from 30 to 70 min.; $P < 0.005$ at 80 min.; $P < 0.05$ at 90 min.; $P < 0.01$ at 100 min.; $P < 0.05$ at 110 and 120 min.).

Plasma glucose also rose discretely, the rise being

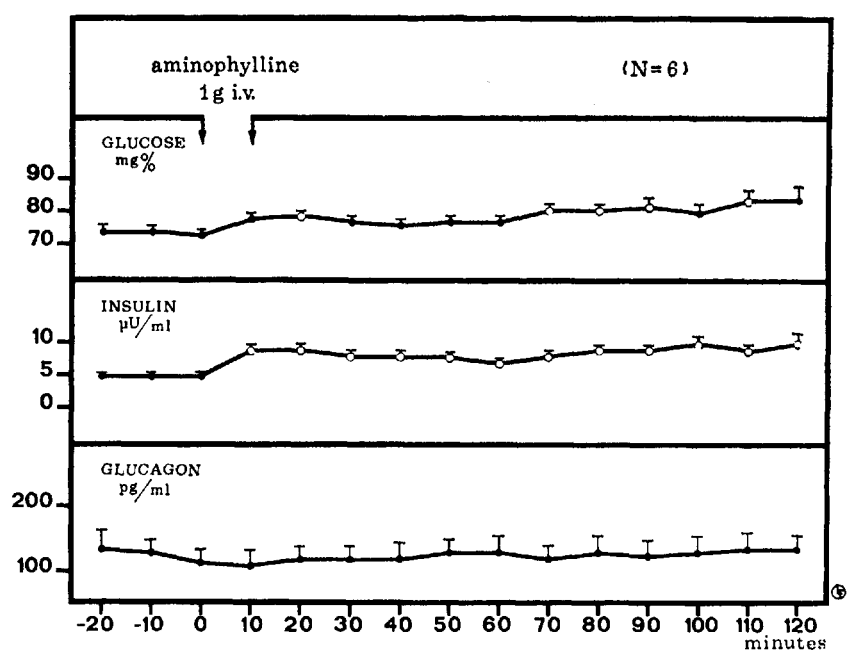

FIGURE 2

Effect of aminophylline on glucagon and insulin secretion in normal subjects. The open circles represent statistically significant differences from the baseline. (Mean ± S.E.M.).

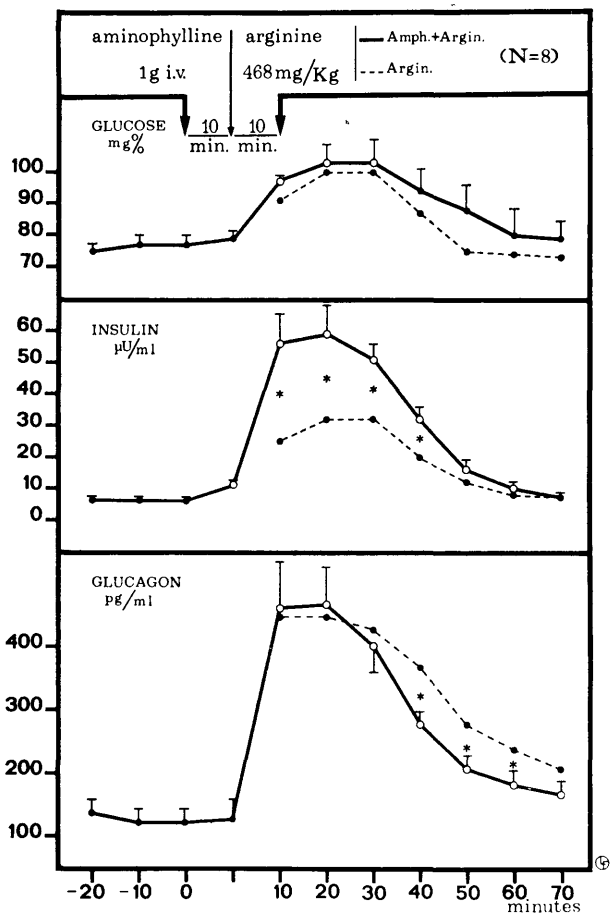

FIG. 3. Effect of aminophylline pretreatment on the glucagon and insulin secretion induced by a high dose of arginine in normal subjects. The open circles represent statistically significant differences from the baseline. The asterisks represent statistically significant differences between the arginine group and the aminophylline-arginine group for a given time. (Mean ± S.E.M.).

significant at 20 min. ($P < 0.05$), 70 min. ($P < 0.005$), 80, 90, and 110 min. ($P < 0.05$).

3. *Effect of aminophylline pretreatment on the glucagon and insulin secretion induced by a high arginine load (468 mg./kg.)* (figure 3).

As can be seen in the figure, the pretreatment with aminophylline did not modify the first part of the glucagon response induced by the high arginine load. The maximal elevation (467 pg./ml. ± 56) was practically identical to that obtained with arginine alone. All points were significantly higher than the baseline ($P < 0.001$ at 10, 20, 30, and 40 min.; $P < 0.01$ at 50 min.; $P < 0.05$ at 60 min.; $P < 0.01$ at 70 min.). However, in the descending portion of the curve, lower glucagon levels were found. The differences of the means were: at 40 min. 94 pg./ml. ($P = 0.025$); at 50 min. 67 pg./ml. ($P = 0.018$); at 60 min. 56 pg./ml. ($P = 0.029$); at 70 min. 42 pg./ml. ($P = 0.053$).

Aminophylline pretreatment markedly potentiated the insulin response to arginine. The differences were statistically significant in all but the last three points of the curve ($P < 0.01$ from 10 to 30 min.; $P < 0.05$ at 40 min.).

Although the glucose curve for post-aminophylline arginine lay above the glucose curve for arginine alone, no statistically significant difference could be detected.

4. Effect of aminophylline pretreatment on the glucagon and insulin secretion induced by a low arginine load (156 mg./kg.) (figure 4).

In this series of experiments no statistically significant difference was observed between the glucagon response to injected arginine in pretreated and non-pretreated subjects. Only the point of maximal elevation (373 pg./ml. ± 86) was significantly different from the baseline ($P < 0.05$).

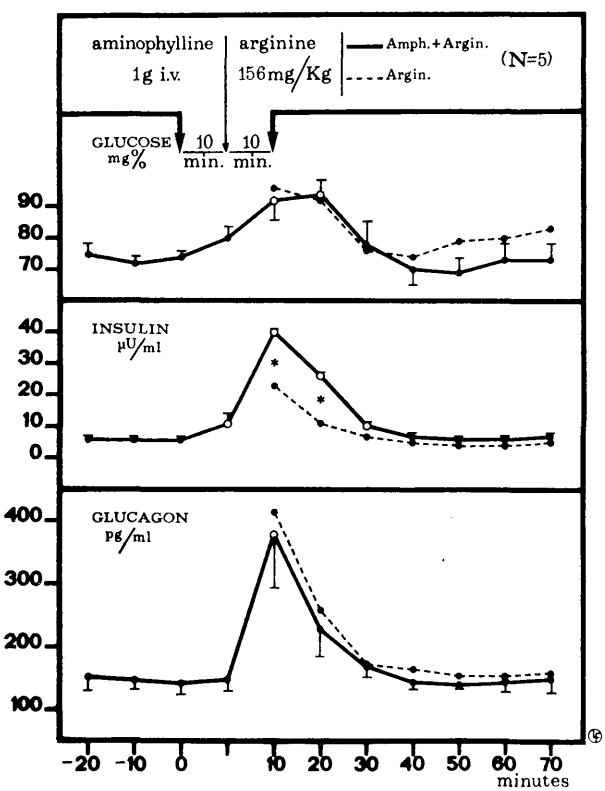

FIG. 4. Effect of aminophylline pretreatment on the glucagon and insulin secretion induced by a low dose of arginine in normal subjects. The open circles represent statistically significant differences from the baseline. The asterisks represent statistically significant differences between the arginine group and the aminophylline-arginine group for a given time. (Mean ± S.E.M.).

As in the high dose group, the insulin values were elevated above those corresponding to the group receiving arginine alone ($P < 0.01$ at 10 and 20 min.). The glucose curve was not altered.

DISCUSSION

This study provides some information about the effect of two different doses of arginine on the secretory response of the alpha cell in humans. The high dose (468 mg./kg.), given as a ten-minute injection, induced a prompt elevation in the plasma glucagon concentration. The maximal peak and the total response seem to be more intense than those reported by Unger et al.[9] for the same amount of arginine when the amino acid was infused over a forty-minute period. With a much smaller dose (156 mg./kg.) a peak of the same magnitude as that of the high load was observed at ten minutes. This seems to indicate that the level of amino acid in blood achieved with the low arginine load is sufficient to elicit an almost maximal acute glucagon secretion which is not ostensibly potentiated by further elevation of the aminoacidemia. After the peak, however, the difference between the stimulatory potentials of the two doses becomes apparent and persists for the duration of the test period. Our results concerning the elevation of the glycemia and insulinemia after arginine are in good agreement with those of others.[9,10]

The intravenous administration of a large dose of aminophylline (1 gm.) did not modify the basal plasma glucagon levels, a finding which supports that of Ensinck et al.[6] Under these circumstances, plasma insulin concentration exhibited a small rise which was associated with a discrete elevation of the glycemia and was maintained for two hours. The stimulatory effect of cyclic AMP and cAMP phosphodiesterase inhibitors on insulin secretion has been extensively reported both in vivo[6,11-13] and in vitro.[2,3,14,15] However, Levine et al.[13] have found a similar stimulatory effect of several adenine nucleotides on insulin release in monkeys and these authors suggest that this effect could be somehow nonspecific for this group of substances. Furthermore, Montague and Cook[16] have recently reported that in isolated rat pancreatic islets the insulinogenic effect of glucose is not accompanied by an increase in the concentration of cAMP, neither in the incubated tissue nor in the medium. Inversely, they were able to increase cAMP concentration in their preparation without a concomitant potentiation of insulin release. Although it is evident that an increase in the intracellular concentration of cAMP stimulates the release of insulin, we believe that a re-evaluation must be made of this effect and of the role of the adenyl cyclase-cAMP system in the secretory mechanism of the beta cell.

When 1 gm. of aminophylline was administered prior to the high arginine load, the glucagon response was similar to that with arginine alone for the first thirty minutes but distinctly lower glucagon values were observed in the last part of the test. Since the administration of aminophylline produces a generalized elevation of the intracellular concentration of cyclic AMP and this has some effect at many tissue sites in the organism, we must regard the diminished glucagon response as a net effect and we cannot attribute it to a direct effect of cAMP on the alpha cell. At the points of lower glucagon concentration, the glycemia curve for the aminophylline pretreated group was not significantly higher than that for the group which did not receive aminophylline, nor was it significantly elevated above the baseline. Therefore, it is unlikely that the lower levels of glucagon were caused by greater hyperglycemia. Although no FFA determinations were carried out, we must take into account that aminophylline enhances lipolysis[6] and that the increased concentration of plasma FFA might have an inhibitory effect on the secretion of glucagon.[17] It cannot be ruled out that the administration of aminophylline could enhance the removal of glucagon from plasma and so, these lower glucagon values would be unrelated to the alpha cell function. It is also possible that the elevation of intracellular cAMP concentration could have an inhibitory effect only on the late phase of glucagon secretion. The experiment in which arginine was employed at the low dose confirms that the aminophylline pretreatment does not alter the early elevation in the circulating glucagon.

Aminophylline pretreatment markedly potentiated the insulin response to both the high and low arginine loads without causing an elevation of the glycemia significantly greater than that produced by arginine alone. Cerasi and Luft[18] did not consistently observe a potentiation of the insulinogenic effect of intravenous glucose by a smaller dose (400 mg.) of aminophylline in non-prediabetic healthy subjects. The discrepancies between our data and those of the above-mentioned authors with regard to the effect of this phosphodiesterase inhibitor on insulin secretion could be due to the different dose and administration thereof or to the stimulus employed or both.

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