Quantitative Determination of the Interaction Between Epinephrine and Various Insulin Releasers in Man

*Suad Efendić, M.D., Rolf Luft, M.D., and Erol Cerasi, M.D.,**
Stockholm

SUMMARY

Epinephrine significantly inhibited glucose-induced insulin release in man, the threshold dose being about 20 ng./kg. per minute. Maximal effect was attained with 60 ng./kg. per minute. In the presence of the threshold epinephrine dose, the dose-response curve for glucose-induced insulin release seemed to be displaced almost in parallel to the right of the control, suggesting competitive inhibition. With the high epinephrine dose the inhibition of insulin release was of a noncompetitive nature.

Epinephrine did not significantly suppress insulin release induced by either arginine or tolbutamide as compared with the effect of these insulinagogues when given at normoglycemia. Only in the case of glucagon did the high epinephrine dose (90 ng./kg. per minute) markedly inhibit the insulinogenic effect of the hormone ($p<0.001$) even when the effect of glucagon at normoglycemia was used as control.

Since glucose exerted synergistic effects on insulin release induced by the other insulinagogues (arginine, glucagon, and tolbutamide), the insulinogenic effect of these insulinagogues in the presence of epinephrine was compared with their effect under hyperglycemic conditions similar to those obtained with epinephrine alone. It was found that epinephrine at a dose of 30 ng./kg. per minute totally inhibited the synergism between the hyperglycemia and the insulinagogues on insulin release. The greater the synergism—as with arginine and glucagon—the larger was the percentage inhibition by epinephrine. A threefold increase in the epinephrine dose further reduced the insulin response.

Our experiments suggest that epinephrine—in the doses used—inhibited the effect of glucose on insulin release. This effect occurred regardless of whether glucose was used as the sole stimulator of insulin release or was used in synergism with such insulinagogues as arginine, tolbutamide, or glucagon. DIABETES *27*:319-26, March, 1978.

Since the classic work of Coore and Randle[1] in vitro and Porte and collaborators[2] in man, the catecholamines have been recognized as powerful inhibitors of insulin release, playing an important role in the modulation of pancreatic islet function under physiologic and pathologic conditions (review[3]).

In man most of the studies regarding catecholamine inhibition of glucose-induced insulin release have been performed with pharmacologic doses of epinephrine. No attempt has been made to analyze the kinetics of the interaction between glucose and epinephrine. Some years ago we reported dose characteristics of the epinephrine inhibition of insulin release and demonstrated that doses as low as 3 ng./kg. per minute, which probably approximate the physiologic response to stress, exerted 50 per cent of the maximal inhibitory effect in man.[4] In the present work, the glucose-epinephrine interaction in man was further analyzed by the use of glucose dose-response studies in the presence of two epinephrine doses. Furthermore, the effect of epinephrine on insulin release induced by other insulinagogues, such as arginine, glucagon, and tolbutamide, was studied.

MATERIALS AND METHODS

The studies were performed on 20 healthy nonobese subjects, mostly blood donors, with a normal glucose tolerance test. Fourteen women and six men with an age range of 25-51 years were used.

The experiments were designed so that each subject

From the Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden.

*Present address: Department of Endocrinology and Metabolism, The Hebrew University Hadassah Medical School, Jerusalem, Israel.

Accepted for publication September 28, 1977.

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served as his own control. Usually different subjects were selected for each set of experiments, but some overlap in the subject material occurred. All experiments were performed in the early morning after an overnight fast and with the subjects resting in supine position. The following experimental designs were used:

Dose dependency of epinephrine inhibition of glucose-induced insulin release. The insulinogenic effect of glucose was determined by performing a glucose infusion test (GIT): the intravenous injection of 500 mg. of glucose per kilogram body weight was followed by a constant-rate infusion (20 mg./kg./min.) for 60 minutes. On different occasions, the GIT was combined with infusions of epinephrine at doses of 20, 30, 60, and 90 ng./kg./minute, respectively, given 10 minutes prior to and again during the GIT.

Modification of the glucose-insulin dose relationship by epinephrine. To establish the glucose-insulin dose relationship, three GITs at the following doses were performed on different occasions: *A*. 250 mg./kg. of glucose injected as a bolus followed by the infusion for 60 minutes of 10 mg./minute; *B*. 500 mg./kg. and 20 mg./kg./minute, respectively; *C*. 1,000 mg./kg. and 40 mg./kg./minute, respectively. When combined with epinephrine, these GITs were performed with the same priming doses of glucose, but the infusion rates were reduced to the following: for *A*, 5 mg./kg./minute; *B*, 10 mg./kg./minute; and *C*, 30 mg./kg./minute. Two doses of epinephrine were used: 20 and 60 ng./kg./minute, given from 10 minutes before until the end of the GIT.

Effect of epinephrine on arginine-induced insulin release. Arginine HCl (Vitrum, Stockholm) was given as a priming dose of 150 mg./kg., followed by the infusion of 10 mg./kg./minute for 30 minutes. The test was repeated twice in combination with the infusion of epinephrine at the doses 30 and 90 ng./kg./minute, given from 30 minutes prior to and again during the arginine infusion. In a fourth experiment, glucose was infused at the rate of 4 mg./kg./minute, 30 minutes before and again during arginine infusion.

Effect of epinephrine on glucagon-induced insulin release. Glucagon (1 mg., Lilly Co., Indianapolis) was given rapidly intravenously (I.V.). In a second series of experiments, glucagon was combined with epinephrine and with glucose, respectively, as described for arginine.

Effect of epinephrine on insulin response to tolbutamide. Tolbutamide (Hoechst AG, Frankfurt/Main) was given I.V. at a dose of 1 gm. The effects of epinephrine, with and without glucose, were tested as described above.

Blood samples were collected in heparinized tubes at the time intervals shown in the figures. Glucose was analyzed in whole blood by the glucose-oxidase method. Plasma insulin was determined by a double-antibody radioimmunoassay.[5] Insulin responses were calculated both as the incremental areas and the insulinogenic indices (insulin areas over glucose areas). The results are given as mean ± S.E.M. of the indicated number of observations.

RESULTS

The dose dependency of the effect of epinephrine on glucose-induced insulin release is shown in table 1. It may be seen that the insulinogenic action of glucose (insulinogenic index) was already reduced by 20 ng./kg./minute of epinephrine, although this effect was not significant. With increasing doses of epinephrine the per cent inhibition was augmented, a maximal effect of 87 per cent being attained at a dose of 60 ng./kg./minute.

TABLE 1

Effect of epinephrine infusion on insulin response to glucose infusion (insulinogenic index)*

Dose of epinephrine (ng./kg./min.)	No. of experiments	Glucose area (mg.min./dl.)	Insulin area (μU.min./ml.×100)	Insulinogenic index	Per cent inhibition of index
0	6	1,526 ± 140	310 ± 44	0.21 ± 0.03	31 ± 22 (N.S.)
20		1,695 ± 199	210 ± 70	0.12 ± 0.03	
0	5	1,584 ± 102	491 ± 120	0.31 ± 0.08	30 ± 9 ($p<0.05$)
30		1,951 ± 169	349 ± 83	0.19 ± 0.07	
0	5	1,438 ± 112	322 ± 69	0.22 ± 0.04	87 ± 1.9 ($p<0.001$)
60		2,221 ± 362	37 ± 17	0.03 ± 0.004	
0	6	1,510 ± 113	414 ± 62	0.28 ± 0.05	87 ± 3.2 ($p<0.001$)
90		2,358 ± 129	73 ± 16	0.03 ± 0.008	

*The insulin response to glucose is expressed as the ratio between the area under the insulin curve with the base at the 0-minute insulin value and the area under the glucose curve with the base at 0 mg./dl. The values presented are insulinogenic index × 100.

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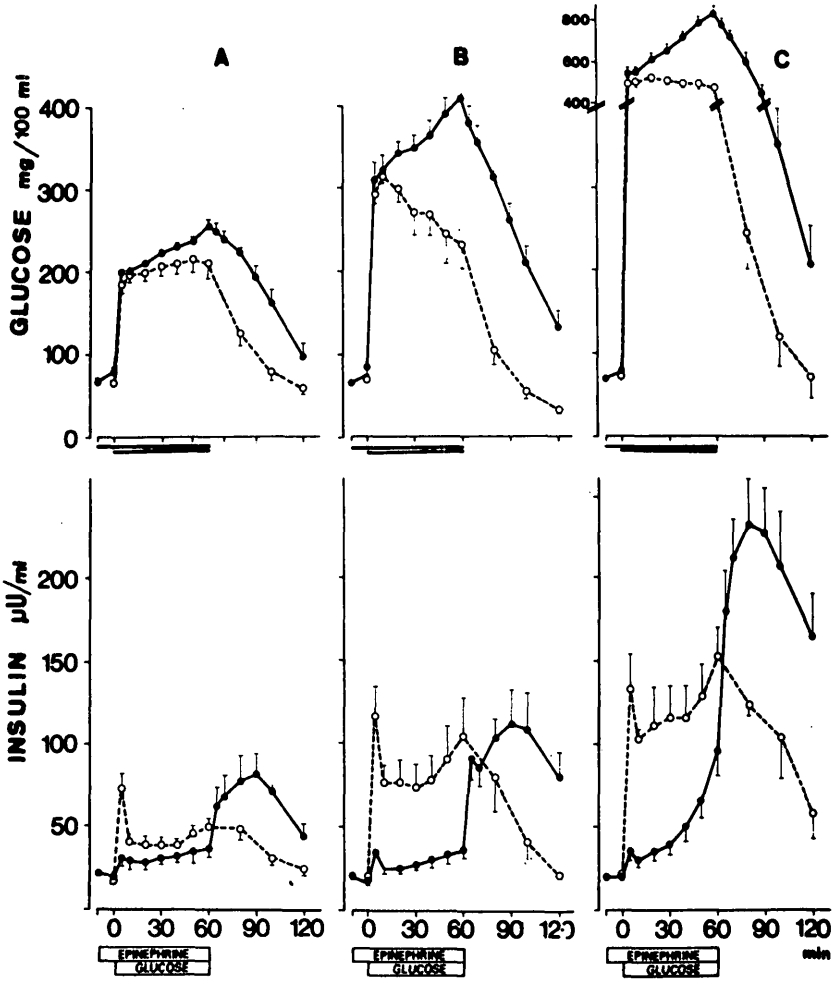

FIGURE 1

Effect of epinephrine (20 ng./kg./minute) on plasma insulin response to varying hyperglycemic stimuli in man. Epinephrine was administered from −10 to 60 minutes and glucose from 0 to 60 minutes. A, B, and C refer to the various glucose doses used (see METHODS). o---o, effect of glucose infusion alone; •—•, effect of combined epinephrine and glucose infusions. Vertical bars denote S.E.M.

The interaction between epinephrine and glucose was studied with two doses of epinephrine: a threshold dose of 20 ng./kg./minute and a maximal one of 60 ng./kg./minute (figures 1-3). The higher blood glucose levels observed throughout the test indicated that the glucose tolerance was already impaired with 20 ng./kg./minute of epinephrine. The effect was more pronounced with the higher dose. These changes mirrored the inhibition of insulin release induced by epinephrine (figures 1 and 2). Under all experimental conditions, the effect was most pronounced during the early phase of insulin release. The hyperglycemia that appeared between 30 and 60 minutes of the GIT resulted in partial reversal of the inhibition of insulin release at later time points. Insulin release was immediately restored after interruption of the epinephrine infusion.

The blood glucose-plasma insulin relationships are presented in figure 3, where the integrated insulin responses (0-60 minutes) are plotted as a function of the corresponding glucose responses. Epinephrine reduced the insulin response at almost all glucose levels. The type of interaction between glucose and epinephrine cannot be defined, since a clear maximal insulin response to glucose was not obtained in these experiments. In the presence of the lower epinephrine dose (figure 3A) the dose-response curve seemed to be displaced almost in parallel to the right of the control one, suggesting competitive inhibition. However, in experiments with the higher dose of epinephrine (figure 3B), the inhibition of insulin response was of a noncompetitive type.

The effect of epinephrine on arginine-induced insulin release is presented in figure 4. Two control studies were performed: one with arginine alone, and another with arginine combined with glucose infusion, in order to mimic the hyperglycemia that follows epinephrine administration. These latter experiments demonstrate the marked synergism between arginine and minimal hyperglycemia (98 ± 4.3 mg./

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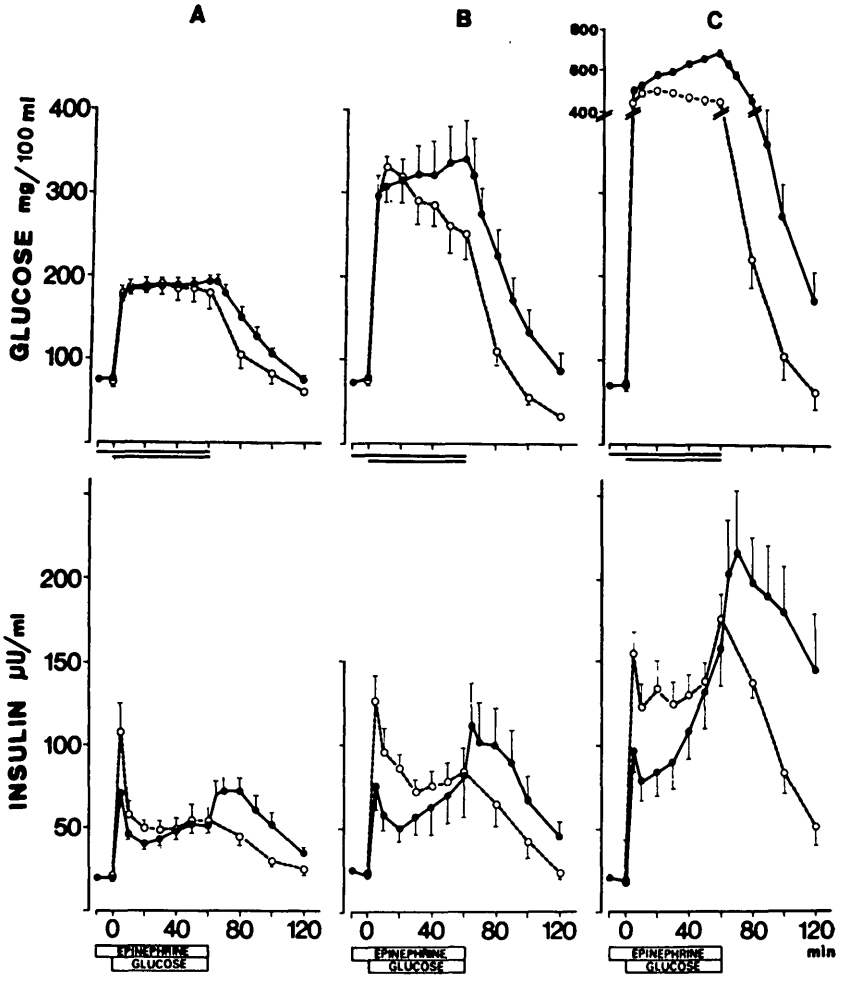

FIGURE 2

Effect of epinephrine (60 ng./kg./minute) on plasma insulin response to varying hyperglycemic stimuli in man. Epinephrine was administered from −10 to 60 minutes and glucose from 0 to 60 minutes. A, B, and C refer to the various glucose doses used (see METHODS). o---o, effect of glucose infusion alone; •—•, effect of combined epinephrine and glucose infusions.

dl.) on insulin release (figure 4). When 30 or 90 ng./kg./minute of epinephrine was given, blood glucose at the initiation of arginine infusion was similar to that achieved during the arginine-glucose experiments (102 ± 7.7 and 104 ± 8.6 mg./100 ml., respectively). The insulin response to arginine was only moderately reduced by epinephrine when compared with the response obtained with arginine alone. However, when compared with the real control (arginine infusion under comparable hyperglycemia), even the lower epinephrine dose exerted prominent inhibition. When the epinephrine infusion was stopped, plasma insulin rose rapidly to high levels, reflecting the uninhibited synergism between hyperglycemia and arginine. The statistical analysis of the results is presented in table 2.

Figure 5 presents the corresponding results with glucagon as the insulin stimulus. The findings were very similar to those with arginine, except that epinephrine seemed to be a somewhat more potent

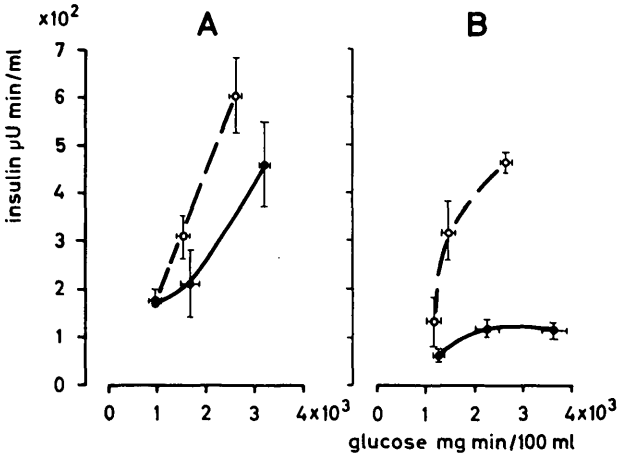

FIG. 3. Modification of the glucose-insulin dose-response relationship by epinephrine 20 ng./kg./minute (A) and 60 ng./kg./minute (B). The response relates the 0-60-minute integrated incremental plasma insulin values to the integrated blood glucose concentrations. Symbols as for figures 1 and 2. The horizontal bars denote S.E.M. for the glucose values.

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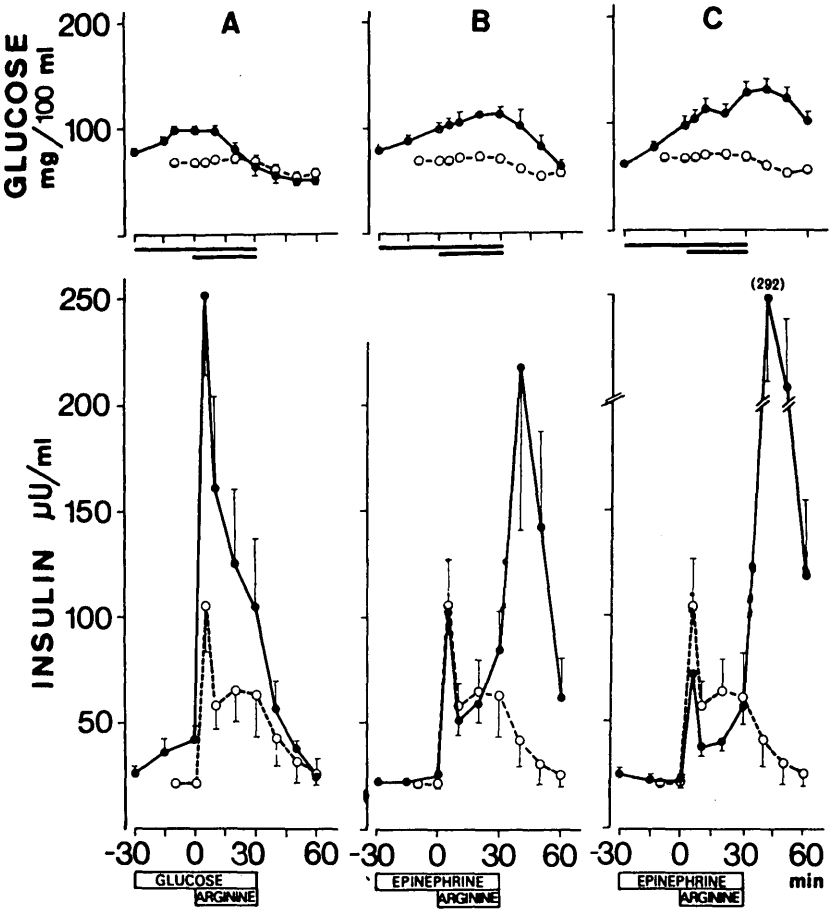

FIGURE 4

Effect of glucose (A) and epinephrine (30 and 90 ng./kg./minute, B and C, respectively) on arginine-induced insulin release. o---o, effect of arginine infusion alone; ●—●, effect of combined arginine and glucose (A) or arginine and epinephrine infusions (B, C). For detailed description of experimental conditions, see METHODS.

TABLE 2

Effect of glucose and epinephrine infusions on insulin release induced by arginine, glucagon, and tolbutamide

Agent	n	Blood glucose at start of test (mg./dl.)	Insulin response (0-30 min., μU.min./ml.)	P-value vs. agent alone	P-value vs. agent + glucose	Per cent change vs. agent alone	Per cent change vs. agent + glucose
Arginine	5	68 ± 1.3	947 ± 74		< 0.001		361 ± 78
Arginine + glucose		99 ± 3.9	3,253 ± 743	< 0.001		361 ± 78	
Arginine + epinephrine (30 ng./kg./min.)		102 ± 7.7	1,059 ± 214	N.S.	< 0.001	114 ± 23	34 ± 4
Arginine + epinephrine (90 ng./kg./min.)		99 ± 7.7	731 ± 138	N.S.	< 0.001	76 ± 12	25 ± 5
Glucagon	5	74 ± 5.0	1,300 ± 195		< 0.005		300 ± 49
Glucagon + glucose		97 ± 4.4	3,637 ± 357	< 0.005		300 ± 49	
Glucagon + epinephrine (30 ng./kg./min.)		95 ± 3.5	1,082 ± 246	N.S.	< 0.001	82 ± 18	28 ± 5
Glucagon + epinephrine (90 ng./kg./min.)		112 ± 9.4	517 ± 88	< 0.001	< 0.001	44 ± 11	14 ± 2
Tolbutamide	7	72 ± 3.9	1,799 ± 241		< 0.05		149 ± 23
Tolbutamide + glucose		106 ± 2.4	2,584 ± 450	< 0.05		149 ± 23	
Tolbutamide + epinephrine (30 ng./kg./min.)		94 ± 3.5	1,891 ± 329	N.S.	< 0.025	109 ± 17	75 ± 8
Tolbutamide + epinephrine (90 ng./kg./min.)		114 ± 4.2	1,290 ± 254	N.S.	< 0.005	74 ± 13	52 ± 11

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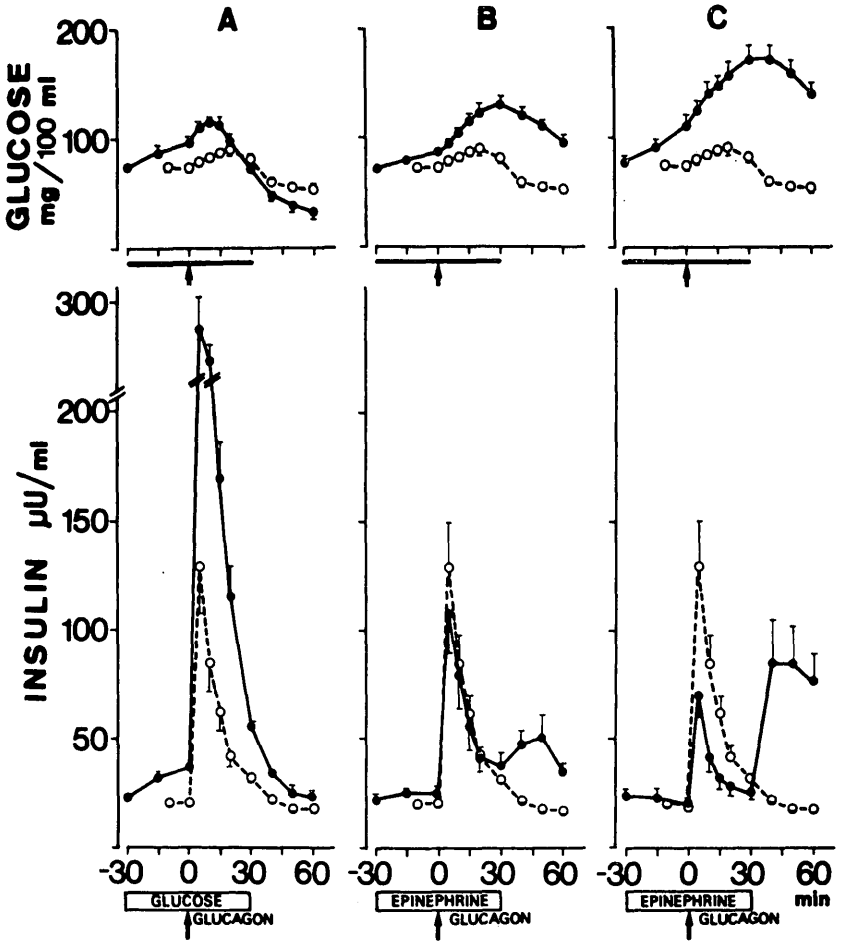

FIGURE 5

Effect of glucose (A) and epinephrine (30 and 90 ng./kg./minute, B and C, respectively) on glucagon (1 mg. I.V.)-induced insulin release. o—o, effect of glucagon alone; •—•, effect of combined glucagon and glucose (A) or glucagon and epinephrine infusions (B, C).

inhibitor of glucagon. Thus, as seen in table 2, the higher dose of epinephrine significantly depressed the insulin response even when compared with glucagon alone, i.e., under normoglycemic conditions. The rebound of plasma insulin after discontinuation of epinephrine infusion was modest.

Contrasting with the previous experiments, the synergism between tolbutamide and the modest elevation of blood glucose (107 ± 3.1 mg./dl.) was much less impressive (figure 6 and table 2). Epinephrine exerted no significant effect on insulin response when compared with tolbutamide alone. In comparison with the insulin response to combined glucose-tolbutamide administration, epinephrine induced significant inhibition. However, it is apparent from table 2 that the effect of epinephrine was less pronounced for tolbutamide than for arginine or glucagon.

DISCUSSION

Epinephrine is a powerful inhibitor of insulin release. For this reason, it is important not to use pharmacologic doses of the catecholamine if the role of epinephrine under physiologic situations is to be understood. In the present experiments, a dose of epinephrine in the order of 30 ng./kg./minute was sufficient to inhibit the insulin response to a major glycemic challenge. In an earlier study,[4] inhibition was obtained with 3 ng./kg./minute and even 1 ng./kg./minute (unpublished observations). These differences suggest that there may exist marked individual variation in sensitivity to epinephrine. The results obtained with the threshold inhibitory dose (20 ng./kg./minute; figure 3A) might be suggestive of competitive inhibition. However, in spite of the very high blood glucose levels that were induced, it was not possible to saturate the insulin response. Therefore, it was difficult to make a kinetic analysis of the inhibition. The findings with a maximal dose of epinephrine (figure 3B), on the other hand, clearly indicate a noncompetitive type of inhibition. Obviously, we cannot exclude the possibility that there may exist different types of inhibition in the presence of low or high doses of epinephrine. To our knowledge, the only in-vitro

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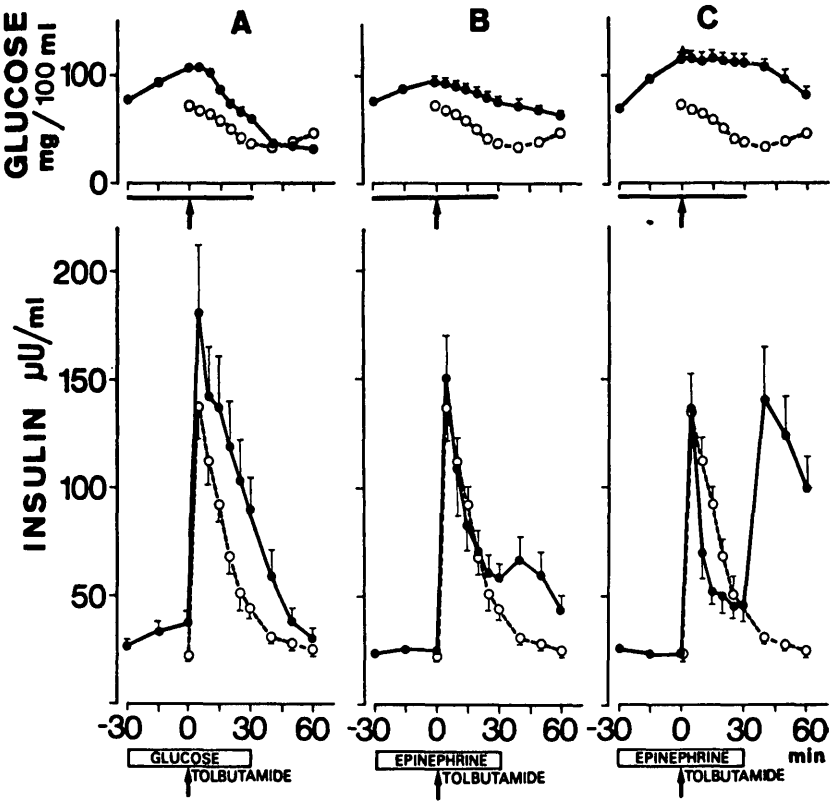

FIGURE 6

Effect of glucose (A) and epinephrine (30 and 90 ng./kg./minute, B and C, respectively) on tolbutamide (1 gm. I.V.)-induced insulin release. o---o, effect of tolbutamide alone; •—•, effect of combined tolbutamide and glucose (A) or tolbutamide and epinephrine infusions (B, C).

study of the kinetics of the epinephrine-glucose interaction is that of Campfield,[7] whose results from perifused rat islets indicated noncompetitive inhibition of insulin release.

It has been shown previously that, when used in high doses in vivo or in vitro, epinephrine also reduces the insulin response to stimulators other than glucose, such as tolbutamide, amino acids, glucagon, theophylline, dibutyryl-cyclic AMP, and short-chain fatty acids (for review, see ref. 7). However, the ability of epinephrine to interfere with the effect of different insulinagogues has not been compared quantitatively. Furthermore, and perhaps for this reason, some confusion seems to exist as to whether, e.g., the effect of arginine on insulin release may be suppressed at all by epinephrine.[8,9] The present work attempted to clarify this point.

The stimulators used here—arginine,[10] glucagon,[11] and tolbutamide[12]—are all known to interact with glucose in enhancing the secretion of insulin in man. However, as is also apparent from figures 4 to 6 and table 2, this synergism is of a different magnitude for arginine and glucagon on the one hand and tolbutamide on the other. Furthermore, while arginine markedly amplified the effect of high glucose,[10] tolbutamide was most efficient at low glucose levels,[12] the synergism together with hyperglycemia (figure 6, table 2) being modest. Therefore, when assaying the in-vivo action of epinephrine on insulin release induced by these agents, the hyperglycemia induced by the catecholamine itself and its synergistic action with the agent used have to be considered.

The results obtained with a dose of epinephrine (30 ng./kg./minute), presumably more in the realm of physiology than pharmacology, clearly indicated that epinephrine totally inhibited the synergism between the minor hyperglycemia and the insulinagogues used (table 2). The greater the degree of synergism (as with arginine and glucagon), the larger was the percentage inhibition of insulin response. A threefold increase in epinephrine further reduced the insulin response. However, a significant inhibition comparable to the response during normoglycemia was obtained only for glucagon. Therefore, it seems clear to us that epinephrine preferentially inhibits the effect of glucose on the beta cell. This effect occurs regardless of whether glucose is used as the sole stimulator of insulin release or participates synergistically with such agents as arginine, tolbutamide, or glucagon. The fact that the insulinogenic activity of these agents requires much higher doses of epinephrine for inhibition (below the response during normoglycemia) may indicate that

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their intrinsic glucose-independent action on the beta cell is resistant to epinephrine and/or that epinephrine is a weaker inhibitor of the synergistic action of basal glucose concentrations. This proposal is supported by the finding that the basal insulin levels, which are influenced by the basal glucose concentration,[13] are difficult to reduce with epinephrine.[4,13] Furthermore, in man the insulin responses to glucagon,[14] arginine,[8] and tolbutamide[15] are negligible when blood glucose is lowered below the fasting level. These studies indicate that any intrinsic glucose-independent insulinogenic effect of these agents must be quite small.

It is not known with certainty how glucose, arginine, glucagon, and tolbutamide, alone or in combination, influence the insulin-secretory mechanisms of the beta cell. The same applies to the inhibitory action of epinephrine. Therefore, it does not seem purposeful to speculate on the reasons for the apparent selectivity of the epinephrine effect on glucose action in the beta cell. Nevertheless, our results clarify some of the confusion in the literature: the in-vivo effect of an inhibitory agent on insulin release must be defined in terms of the prevailing synergism with glucose. This is more important when such hyperglycemia-inducing agents as epinephrine are used. A further practical conclusion is that minor to moderate stress, while effectively blocking the insulin response to glucose, may not significantly influence the stimulation of the islets by such other agents as the oral hypoglycemic drugs.

ACKNOWLEDGMENTS

The authors are grateful to Mrs. Kerstin Waldelöf, Mrs. Christina Thornqvist, and Mrs. Anneli Nyberg for devoted technical assistance and to Mrs. Ulla-Britt Nilsson and Miss Britt-Marie Witasp for secretarial help. These studies were supported by grants from the Swedish Medical Research Council (contracts nos. B77-19X-0034-13B, B77-19X-04540-03A), the Swedish Diabetes Association, and the Nordic Insulin Foundation.

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