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**Research Article**

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# Interactions of Acetylcholine and Epinephrine on the Dynamics of Insulin Release in Vitro

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Abstract An in vitro system for perifusion of rat pancreatic islets has been utilized to define the effects of epinephrine on acetylcholine-induced insulin release over varying concentrations of the two agents. Perifusion of islets with epinephrine before challenge with acetylcholine produced marked enhancement of both phases of cholinergically induced insulin release; enhancement of the first phase being more marked with increase in acetylcholine concentration and the converse being observed with the second phase. Perifusion of islets with epinephrine during stimulation with acetylcholine produced inhibition of insulin release, an effect dependent upon the concentration of epinephrine and of acetylcholine. There was an order of difference in the acetylcholine concentration needed to overcome significant epinephrine-mediated inhibition of the first phase of insulin release ($5 \times 10^{-4}$ $\mu$g/ml) and that needed to overcome inhibition of the second phase ($5 \times 10^{-3}$ $\mu$g/ml). Comparison of the effects of various concentrations of epinephrine on glucose- and acetyl-choline-induced insulin release revealed that epinephrine was a less potent inhibitor of the first phase of acetylcholine-induced insulin release than of the first phase of glucose-induced insulin release. These data provide some insight into the potential interactions between cholinergic and adrenergic autonomic systems in modifying insulin release.

## INTRODUCTION

The often reciprocal effects mediated through the sympathetic and parasympathetic nervous systems form the basis for much of our understanding regarding the role of the autonomic nervous system in contributing to homeostatic mechanisms in general. Teleologically, this concept should be applicable to metabolic homeostasis (1). Over the last few decades, data has accumulated to indicate the central mechanisms through which an organism may control its metabolic homeostasis with respect to the external environment (1–15). Similarly, the same or similar central areas can exert control over the internal metabolic milieu (16–22). Pathways through which these latter effects could be mediated include direct autonomic nervous system control over tissue metabolism (23–26) and direct control mediated through autonomic nervous system modification of hormonal release (18–21, 23, 26–31).

Pertinent to this report are the observations regarding the effect of adrenergic agents in inhibiting insulin release via $\alpha$-adrenergic receptors; stimulating release, via $\beta$-adrenergic receptors (32), or; "priming" B cells for enhanced responsiveness to subsequent glucose challenge (31, 33) and similar data indicating the ability of cholinergic mechanisms to stimulate insulin release (29, 34–38). Thus, much is known about the independent effects of these autonomic systems on insulin release. However, little is known about the interelationship between these two systems as they affect insulin release. This concern becomes pertinent in light of present concepts of neural control of metabolic processes outlined above.

In this study an attempt has been made to define the interactions between the parasympathetic and the adrenergic systems in modifying the dynamics of insulin release. An in vitro perifusion system has been used in which biphasic insulin release in response to constant challenge by glucose (38) and(or) acetylcholine (39) has been demonstrated. The following questions were considered. Does epinephrine inhibit cholinergically induced insulin release? Does epinephrine prestimulation enhance the insulin release in response to subsequent acetylcholine stimulation? Are either the prestimulatory (priming) or inhibitory effects of epinephrine modified by the concentration of acetylcholine used? Are there differences between the two phases of cholinergically induced insulin release in their response to the effects

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of epinephrine? Finally, is epinephrine as effective in inhibiting and priming cholinergically induced insulin release as it is in inhibiting and priming glucose-induced insulin release?

## METHODS

Islets were obtained from fasting male Wistar rats weighing from 200 to 250 grams by a previously described modification (40) of the collagenase method of Lacy and Kostianovsky (41). Two sets of 40 islets were utilized in each experiment allowing for simultaneous performance of control and test experiments from the same pool of islets. Islets were perifused utilizing a previously described perifusion apparatus (31, 33, 39, 42) which permits appropriate temperature control (thermostate-pump-water bath), gassing of buffer (with 5% $CO_2$, 95% $O_2$) and continuous collection of perifusate in a fraction collector housed in a cold box. Collected samples were frozen at the end of each experiment for assay of insulin content by a modification of the Hales and Randle double-antibody method (43).

Islets (40 per chamber) were perifused at buffer flow rates of 2.2–2.4 ml/min in both the prestimulation period of 25 min and the subsequent test or control period of 60 min with Krebs-Ringer bicarbonate containing 0.5 g/100 ml albumin and 50 mg/100 ml glucose to which was added: (*a*) acetylcholine in concentrations from $5 \times 10^{-5}$ μg/ml (0.27 nM) to $10^{-1}$ μg/ml (5.4 μM) during the test period, or (*b*) with epinephrine 0.5 μg/ml (2.3 μM) plus acetylcholine in varying concentrations during the test period, or (*c*) epinephrine 0.5 μg/ml during the prestimulation period followed by acetylcholine in varying concentrations during the test period, or (*d*) with epinephrine 0.5 μg/ml added during both the prestimulation and the test periods, or (*e*) with no additions, series (*d*) and (*e*) representing appropriate controls for series (*a*), (*b*), and (*c*).

A further series of experiments were performed utilizing epinephrine in concentrations of 0.01 → 0.5 μg/ml (0.11–2.3 μM) in either the prestimulation buffer and(or) the stimulation buffer in the presence of either 0.01 μg/ml acetylcholine (54 nM) or 300 mg/100 ml glucose (16.4 mM) as the stimulating agent.

In each experimental series patterns of insulin release were defined by measuring the insulin concentration in all samples of the perifusate over the first 10 min and every fifth sample thereafter. The perifusate was collected in aliquots representing 1-min intervals for the latter 5 min of the prestimulation period, 30-s intervals for the initial 10 min of the stimulating (test or control) period and 1-min intervals for the remainder of this second period. All experiments were performed at least six times. Total amounts of insulin released in each of the two phases were defined by reference to the insulin concentration; time plots either by addition for the acute (first phase, $T_1$) response where all samples were measured, or by calculation (from the area subtended by the insulin release: time curve) for the sec-

TABLE I

*Effect of Epinephrine on the Two Phases of Acetylcholine-Induced Insulin Release and Variable Acetylcholine Concentrations*

| | | Prestimulation Epi | Stimulation ACh | Stimulation Epi | Insulin release $T_1$ | Insulin release $T_2$ | vs. (a)* $T_1$ | vs. (a)* $T_2$ |
|---|---|---|---|---|---|---|---|---|
| | *n* | *0.5 μg/ml* | *μg/ml* | *0.5 μg/ml* | *ng±SEM* | | *P* | |
| (a) | 12 | − | 0 | − | 0.9±0.1 | 4.1±1 | — | — |
| (b) | 6 | − | 0 | + | 0.8±0.1 | 3.8±0.8 | NS | NS |
| (c) | 6 | + | 0 | − | 0.9±0.2 | 4.0±1 | NS | NS |
| (a) | 6 | − | $5 \times 10^{-5}$ | − | 7.7±0.1 | 34.7±0.8 | — | — |
| (b) | 6 | − | $5 \times 10^{-5}$ | + | 5.4±0.2 | 21.4±1.0 | <0.001 | <0.001 |
| (c) | 6 | + | $5 \times 10^{-5}$ | − | 8.9±0.3 | 42.8±2.5 | <0.05 | <0.05 |
| (a) | 6 | − | $5 \times 10^{-4}$ | − | 9.6±0.3 | 99.4±0.7 | — | — |
| (b) | 6 | − | $5 \times 10^{-4}$ | + | 8.7±0.2 | 72.6±0.7 | NS | <0.01 |
| (c) | 6 | + | $5 \times 10^{-4}$ | − | 12.4±0.2 | 115.8±1.6 | <0.05 | <0.05 |
| (a) | 6 | − | $1 \times 10^{-3}$ | − | 10.6±0.4 | 132.5±1.3 | — | — |
| (b) | 6 | − | $1 \times 10^{-3}$ | + | 9.5±0.1 | 117.0±1.1 | NS | <0.01 |
| (c) | 6 | + | $1 \times 10^{-3}$ | − | 14.8±0.1 | 145.7±1.0 | <0.001 | <0.01 |
| (a) | 10 | − | $1 \times 10^{-2}$ | − | 14.4±0.3 | 173.3±3.2 | — | — |
| (b) | 6 | − | $1 \times 10^{-2}$ | + | 12.4±0.1 | 162.0±0.8 | NS | <0.05 |
| (c) | 6 | + | $1 \times 10^{-2}$ | − | 18.4±0.3 | 188.3±4.6 | <0.001 | <0.01 |
| (a) | 6 | − | $1 \times 10^{-1}$ | − | 55.7±2 | 311±7.5 | — | — |
| (b) | 6 | − | $1 \times 10^{-1}$ | + | 60.9±3.2 | 316±5.2 | NS | NS |
| (c) | 6 | + | $1 \times 10^{-1}$ | − | 74.9±4.0 | 342±8.0 | <0.001 | <0.01 |

(a), Stimulation with acetylcholine alone (ACh); (b), stimulation with ACh in the presence of epinephrine (Epi); (c), stimulation with ACh after prestimulation with Epi.

* In all Tables, vs. = versus; i.e., statistical comparison of (b), (c), or (d) with (a) in each series.

TABLE II

*Effect of Epinephrine on Acetylcholine-Induced Insulin Release and Variable Epinephrine Concentrations*

| | | Prestimulation Epi | Stimulation | | Insulin release | | vs. (a) | |
|---|---|---|---|---|---|---|---|---|
| | | | ACh | Epi | $T_1$ | $T_2$ | $T_1$ | $T_2$ |
| | *n* | *0.5 μg/ml* | *0.02 μg/ml* | *0.5 μg/ml* | *ng±SEM* | | *P* | |
| (a) | 6 | — | + | — | 14.4±0.3 | 173.3±3.2 | — | — |
| (b) | 6 | — | + | 0.5 | 12.4±0.1 | 162.0±0.8 | NS | <0.05 |
| (c) | 6 | 0.5 | + | — | 18.4±0.3 | 188.3±4.6 | <0.001 | <0.01 |
| (d) | 6 | 0.5 | + | 0.5 | 15.2±0.3 | 175.1±3.1 | NS | NS |
| (a) | 6 | — | + | — | 14.4±0.3 | 173.3±3.2 | — | — |
| (b) | 6 | — | + | 0.1 | 12.5±0.2 | 153.8±2.0 | NS | <0.05 |
| (c) | 6 | 0.1 | + | — | 18.6±0.3 | 206.6±2.8 | <0.001 | <0.01 |
| (d) | 6 | 0.1 | + | 0.1 | 16.0±0.3 | 179.4±2.6 | NS | NS |
| (a) | 8 | — | + | — | 14.4±0.3 | 173.2±3.2 | — | — |
| (b) | 6 | — | + | 0.01 | 14.4±0.2 | 169.7±3.0 | NS | NS |
| (c) | 6 | 0.01 | + | — | 15.7±0.2 | 184.7±2.9 | NS | NS |
| (d) | 6 | 0.01 | + | 0.01 | 14.7±0.3 | 164.4±2.8 | NS | NS |

(a)–(c), As in Table I; (d), epinephrine present through both the prestimulation and the stimulation periods. ACh 0.02 μg/ml = 54 nM.

ondary response where every fifth sample was measured (second phase, $T_2$).

## RESULTS

Table I summarizes the insulin responses obtained during acetylocholine stimulation over the concentration range of $5 \times 10^{-5}$ μg/ml (0.27 nM) to $1 \times 10^{-1}$ μg/ml (5.4 μM) in the absence of epinephrine and when a fixed concentration 0.5 μg/ml (2.3 μM) was added to the buffer either before or during acetylcholine stimulation. The effect of varying the concentration of epinephrine added to either the prestimulation buffer or the stimulation buffer, or both, on the insulin released in response to acetylcholine or glucose challenge, is summarized in Tables II and III, respectively. In all tables series (a) represents studies in the absence of epinephrine, series (b) represents studies with epinephrine added together with the insulinogogue, series (c) represents epinephrine added to the prestimulation media alone, and series (d) represents epinephrine present in both the prestimulation and the stimulation buffers. Statistical comparisons in Tables

TABLE III

*Effect of Epinephrine on the Two Phases of Glucose-Induced Insulin Release and Variable Epinephrine Concentrations*

| | | Prestimulation Epi | Stimulation | | Insulin release | | vs. (a) | |
|---|---|---|---|---|---|---|---|---|
| | | | G | Epi | $T_1$ | $T_2$ | $T_1$ | $T_2$ |
| | *n* | *μg/ml* | *300 mg/dl* | *μg/ml* | *ng±SEM* | | *P* | |
| (a) | 16 | — | + | — | 14.2±0.3 | 204.5±5.6 | — | — |
| (b) | 6 | — | + | 0.5 | 7.0±0.2 | 162±4.8 | <0.001 | <0.01 |
| (c) | 6 | 0.5 | + | — | 20.1±2.2 | 267±5.6 | <0.001 | <0.01 |
| (d) | 6 | 0.5 | + | 0.5 | 14.9±0.4 | 210±5.1 | NS | NS |
| (a) | 16 | — | + | — | 14.2±0.3 | 204.5±5.6 | — | — |
| (b) | 6 | — | + | 0.1 | 10.8±0.3 | 184.7±3.7 | <0.05 | <0.05 |
| (c) | 6 | 0.1 | + | — | 16.0±0.5 | 215.2±4.8 | <0.05 | <NS |
| (d) | 6 | 0.1 | + | 0.1 | 14.0±0.3 | 208±4.6 | NS | NS |
| (a) | 16 | — | + | — | 14.2±0.3 | 204.5±5.6 | — | — |
| (b) | 6 | — | + | 0.01 | 13.5±0.3 | 201±4.6 | NS | NS |
| (c) | 6 | 0.01 | + | — | 14.6±0.2 | 207±5.2 | NS | NS |
| (d) | 6 | 0.01 | + | 0.01 | 14.4±0.3 | 202±4.1 | NS | NS |

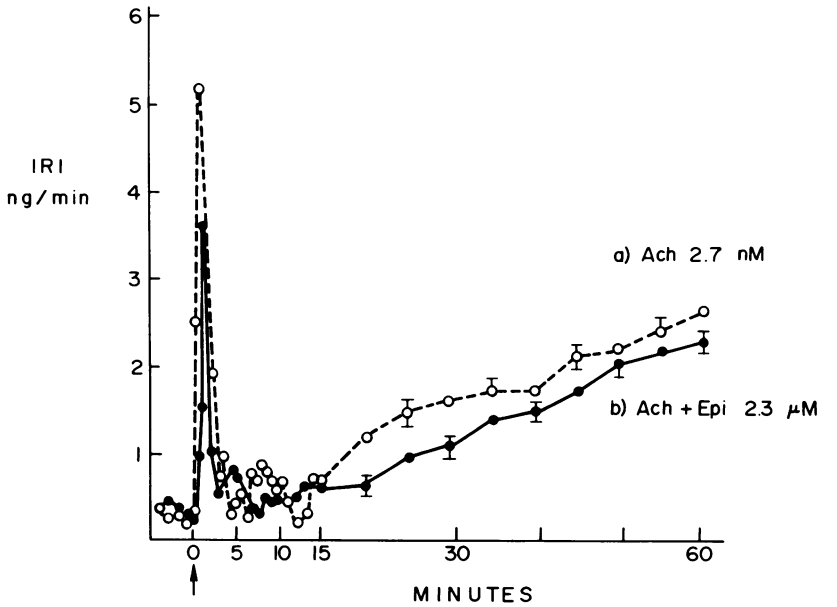


FIGURE 1 Effect of Epi on ACh-induced insulin release when Epi is present only during the period of ACh challenge. Stimulation with ACh for 60 min commenced at time zero after a preliminary perifusion period of 25 min. The dashed line indicates the response to ACh alone, series (a), and the solid line the response to ACh in the presence of Epi, series (b). Each point and bar represents the mean and SEM of at least six determinations ACh 2.7 nM $\equiv 5\times 10^{-4}$ μg/ml; Epi 2.3 μM ≡ 0.5 μg/ml.

I–III compare the response in the presence of epinephrine (series b-d) with the responses obtained in the absence of epinephrine (series a).

Addition of epinephrine during stimulation with acetylcholine (Fig. 1, Tables I and II) produced dose-dependent inhibition of insulin release. Both phases of acetylcholine induced biphasic insulin release were affected. However, the inhibitory effect of 0.5 μg/ml (2.3 μM) epinephrine on either phase was much less marked with higher concentrations of acetylcholine (Fig. 3). There was a twofold difference in the acetylcholine concentration needed to overcome significant epinephrine-mediated inhibition of the first phase of insulin release, achieved at $5 \times 10^{-4}$ μg/ml (2.7 nM) acetylcholine and that concentration of acetylcholine, $1 \times 10^{-3}$ μg/ml (5.4 nM) required to overcome epinephrine-mediated inhibition of the second phase of acetylcholine-induced insulin release.

As indicated in Fig. 2 and Tables I and II (series c), perifusion of islets with epinephrine before subsequent acetylcholine stimulation enhanced both phases of cholinergically induced insulin release. This effect was dependent upon the concentration of acetylcholine used (Table I and Fig. 3) and differed for the two phases. The percentage of enhancement of the first phase of acetylcholine-induced insulin release attributable to prior exposure to epinephrine tended to increase with increase in the concentration of acetylcholine (Fig. 3). The converse was true for the second phase of acetylcholine-induced insulin release, such that at high acetylcholine concentrations prior exposure to 0.5 μg/ml (2.3 μM) epinephrine had minimal or no effect upon the second phase of acetylcholine-induced insulin release (Fig. 3).

The effect of maintaining epinephrine throughout both the prestimulation and stimulation periods is indicated in Table II. There was no evidence for any effect on acetylcholine-induced insulin release under these circumstances.

The effect of varying the epinephrine concentration on the ability of epinephrine to either enhance (when added to the prestimulation buffer) or inhibit (when added with acetylcholine) acetylcholine-induced insulin release, is summarized in Table II. It is apparent that while the effect of epinephrine is dose dependent over the concentration range 0.01–0.5 μg/ml (0.11–2.3 μM) there is very little difference in the effects of 0.1 and 0.5 μg/ml of epinephrine on either enhancing or inhibiting acetylcholine-induced insulin release.

Table III summarizes the data obtained when variable concentrations of epinephrine are added either before, or during stimulation of islets with 300 mg/100 ml glucose. The qualitative effects of epinephrine on glucose-induced insulin release can be seen to be similar to the effects of epinephrine on acetylcholine-induced insulin release. With these concentrations of glucose and acetylcholine comparable amounts of insulin are released in response to either secretogogue in the absence of epi-

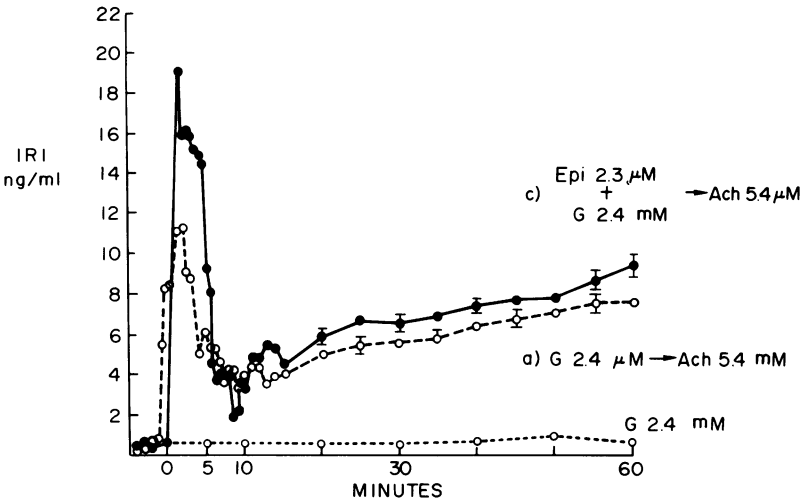


FIGURE 2 Effect of Epi on the dynamics of ACh-induced insulin release when Epi is added only to the prestimulation buffer (which perifuses the islets for 25 min before onset of ACh challenge at time zero). The upper dashed line represents the response to ACh in the absence of Epi prestimulation, series (a); the solid line represents the response to ACh of islets subjected to Epi prestimulation, series (c). The lower dashed line refers to insulin response to the presence of 2.4 mM glucose. Points and bars are means of SEM's of at least six determinations. ACh 5.4 $\mu$M $\equiv 1 \times 10^{-3}$ $\mu$g/ml.

nephrine. Moreover, as illustrated in Fig. 4, differences in epinephrine responsiveness between acetylcholine- and glucose-induced insulin release are observed as the concentration of epinephrine is changed. In particular, epinephrine at all concentrations was a less potent inhibitor of the first phase of insulin release induced by acetylcholine than of the first phase of glucose-induced insulin release. A similar differential effect of epinephrine was observed on the second phase of acetylcholine- and glucose-induced insulin release at the higher epinephrine concentration (0.5 $\mu$g/ml). In contrast to the effect of epinephrine on acetylcholine-induced insulin release, a marked difference in the effectiveness of 0.1 vs. 0.5 $\mu$g/ml epinephrine was demonstrated with glucose-induced insulin release. A similar difference between acetylcholine and glucose-induced insulin release was observed in the dose response to epinephrine when epinephrine was added before stimulation with the insulinogogue. However, under these circumstances the high epinephrine concentration produced greater enhancement of both first and second phase insulin responses to glucose than enhancement of acetylcholine-induced insulin release, whereas, at the lower epinephrine concentration the converse was observed. That is, a fivefold change in epinephrine concentration had little effect on the action of epinephrine on acetylcholine-induced insulin release, but did have a significant effect on glucose-induced insulin release. Finally, epinephrine added before stimulation of islets with either glucose or acetylcholine, produced a more marked enhancement of the primary than of the secondary insulin release response.

## DISCUSSION

In this study, an in vitro system was utilized to assess the interrelationship between epinephrine and acetylcholine (ACh) on the dynamics of insulin release and to compare the effects of epinephrine on substrate (glucose) and cholinergically induced insulin release. The concentrations of ACh used (ranging from 0.27 nM to 5.4 $\mu$M) are at, or below, the dissociation constant for acetylcholine ($10^{-6}$ M) and thus, are assumed to be within the physiologically significant range. The concentration of glucose utilized (16.4 mm) is approximately half that needed for maximal stimulation in most in vitro systems and the concentration of epinephrine ($10^{-6}$ M) is that which produces significant, but not complete, suppression of glucose-induced insulin release in vitro; that is, a concentration which in vitro effectively mimics demonstrable in vivo effects.

The results obtained in this study indicate that epinephrine has variable effects on acetylcholine-induced insulin release, which are dependent on both the concentration of epinephrine utilized, and on the temporal relationship between the exposure to epinephrine and the challenge with acetylcholine, with inhibition occurring when epinephrine is added with the acetylcholine. A cooperative effect between epinephrine and acetylcholine was observed when islets were exposed to epinephrine before acetylcholine challenge. That is, preincubation with epinephrine resulted in enhancement of the insulin response to subsequent acetylcholine challenge. No apparent effect was observed on acetylcholine-induced in-

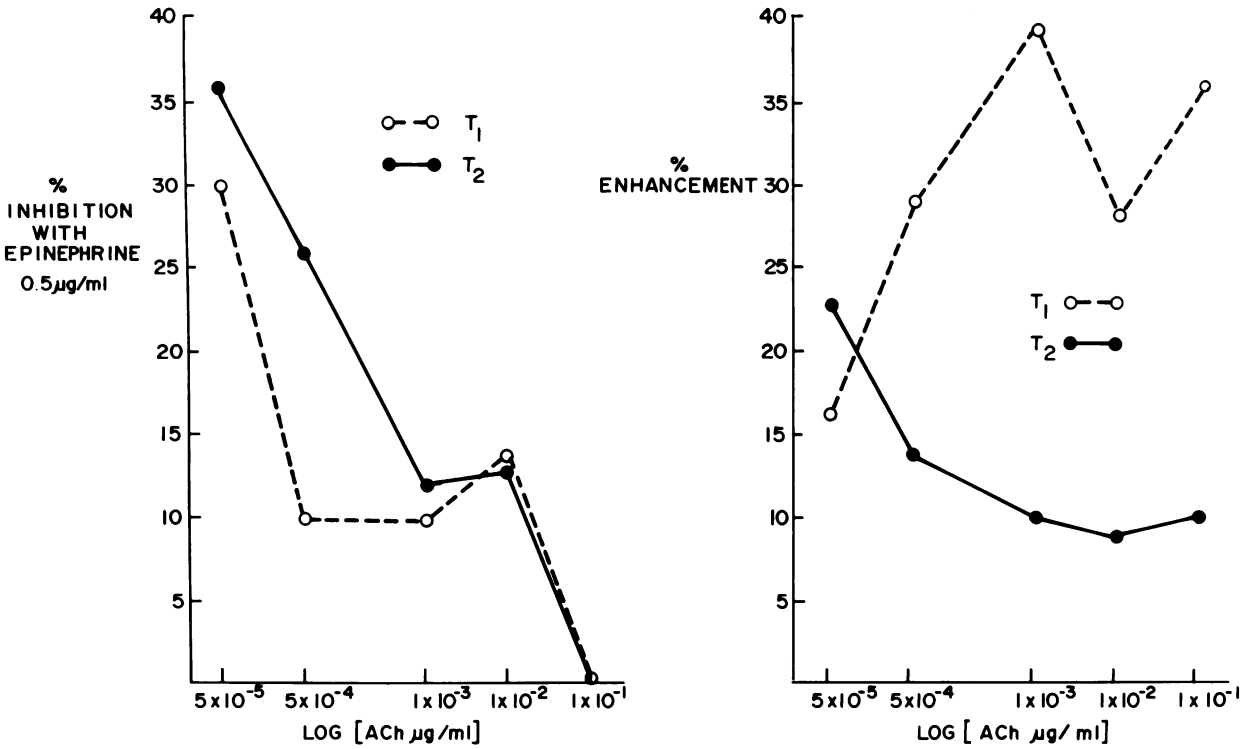


FIGURE 3 Effect of ACh concentration on the inhibition of ACh-induced insulin release induced by the simultaneous presence of Epi (left panel) and on the enhancement of ACh-induced insulin release resulting from prior exposure of islets to Epi (right panel). $T_1$ and $T_2$ represent the total amount of insulin released in the first and second phases of ACh-induced insulin release (see text and Figs. 1 and 2). Epi 0.5 μg/ml ≡ 2.3 μM.

sulin release when islets were continuously exposed to epinephrine before and during acetylcholine challenge. In these above respects, the effects of epinephrine on insulin responses to acetylcholine are qualitatively comparable to its effects on glucose-induced insulin release.

The data provide the basis for a more detailed analysis of this phenomenon. This analysis will consider the following. First, the relative effectiveness of epinephrine in inhibiting either the first of the second phase of acetylcholine-induced insulin release and the effect of variation in ACh and epinephrine (Epi) concentration on this effect. Second, the effect of changes in epinephrine and acetylcholine concentration on the cooperative (priming effect) and the relative effectiveness of such priming on the two phases of acetylcholine-induced insulin release. Third, the relative effectiveness of epinephrine in modifying ACh and glucose-induced insulin release.

The inhibition by epinephrine of the first phase of insulin release induced by acetylcholine could be overcome by a concentration of acetylcholine at least one order of magnitude less than that concentration of acetylcholine necessary to overcome the inhibitory effect of epinephrine on the second phase of acetylcholine-induced insulin release. Further, when the acetylcholine concentration approached the binding constant for ACh (1 μM), the inhibitory effect of a comparable, but higher concentration of epinephrine (2.7 μM) was abolished. That is, the qualitative and quantitative integrity of the insulin release response to acetycholine can readily be attained in the face of epinephrine by increasing the concentration of acetylcholine within the presumed physiological range. In particular, the acute responsiveness of the insulin release mechanism is maintained (first phase insulin release) at lower ACh concentrations than the later phase in the face of epinephrine inhibition. Thus, the effector mechanism exists for ensuring appropriate acute (first phase) insulin release even in the face of enhanced adrenergic tone.

Preincubation with epinephrine before stimulation with acetylcholine produced enhancement of both phases of ACh-induced insulin release and, apart from the response to the lowest concentration of ACh used, $5 \times 10^{-5}$ μg/ml (0.27 nM) produced a greater degree of enhancement of the first, than of the second phase of ACh induced insulin release over a wide range of ACh concentrations. That is, Epi produced qualitative effects on

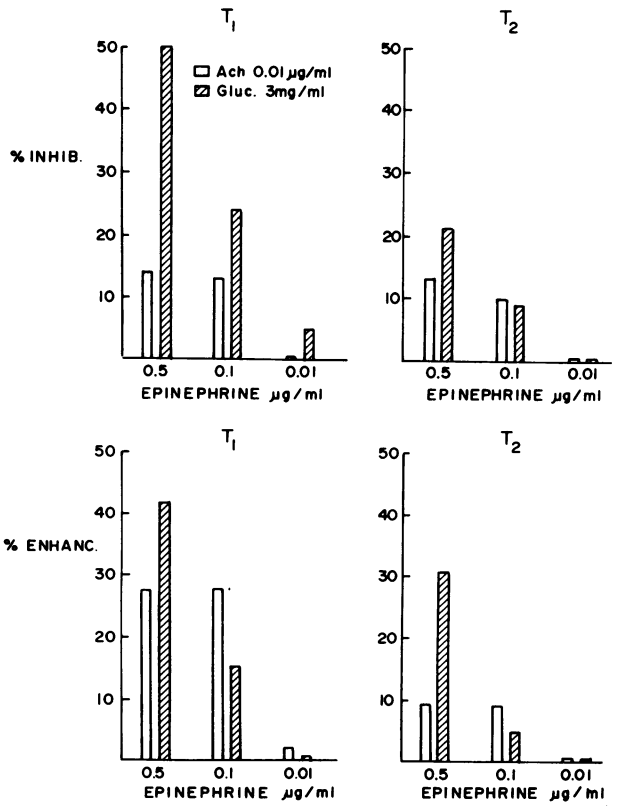


FIGURE 4 Effect of change in Epi concentration on its ability to modify ACh- and glucose-induced insulin release. The upper panel illustrates the percentage inhibition of glucose-induced (hatched bars) and ACh-induced (clear bars) insulin release when Epi is added only during the period of ACh stimulation. The lower panel illustrates the percentage enhancement of insulin release attained by exposing islets to Epi during the 25 min prestimulation period before stimulation with ACh.

ACh-induced insulin release similar to that observed when glucose is used as the insulinogogue. Thus, the results of epinephrine priming in vitro indicate that appropriately timed adrenergic stimulation can provide a mechanism for maintaining the integrity of the acute insulin response of the islet to subsequent stimulation, whether by substrate (e.g., food), or by acethycholine (e.g., reflectly induced release).

In contrast to the "priming" and "inhibiting" effects of epinephrine on ACh- and glucose-induced insulin release, the addition of epinephrine during both the stimulation and the prestimulation periods, does not influence the insulin response to either ACh or glucose, either quantitatively or qualitatively. While it must be assumed that the apparent net cancellation of priming and inhibitory effects is the result of an accident of experimental design (that is the duration of preincubation and stimulatory periods), the observation did hold true over a range of epinephrine concentrations. Thus, it would appear from this data that for optimal insulin release, that adrenergic tone, acting specifically on B-cell islets, should be maximal during nonsecretory phases (e.g., in pre- or interprandial) periods and minimal during periods of insulin response (e.g., to feeding, whether the response be induced reflexly [ACh], or by a rise in substrate availability, glucose). An alternative, but not mutually exclusive, explanation would be that rate of decrement in intensity of adrenergic stimulation may be important in producing the priming effect. If the reverse were true, insulin release would be inhibited; if adrenergic "tone " remained unaltered, then the insulin release response would be similar to that of adrenergically denervated islets (that is, less than that obtained with "appropriately" innervated or "primed" islets). The existence of "tonic" adrenergic stimulation to B cells in fasting state is indicated by the effect of chemical sympathectomy which produces elevated fasting insulin levels, despite attenuated insulin release in response to challenge (44).

Comparison of the effects of epinephrine on ACh-induced insulin release and on glucose-induced insulin release reveals a number of potentially important points. First, epinephrine appears to be a more potent inhibitor of glucose-induced insulin release than of ACh-induced insulin release. This is illustrated by the fact that 0.5 µg/ml (2.7 µM) epinephrine produces 50% inhibition of the first phase and 24% inhibition of the second phase of glucose-induced insulin release (using 16.4 mM glucose) and only 14% and 13% inhibition respectively of the first and second phases of acetylcholine-induced insulin release (using 0.01 µg/ml ACh which induces approximately the same quantitative and qualitative insulin release pattern as 16.4 mM glucose). Second, the responses of glucose-induced insulin release to adrenergic "priming" and to adrenergic inhibition are more responsive to changes in epinephrine concentration than are the responses induced by ACh. That is, marked changes in enhancement and inhibition are observed with glucose-induced insulin release over the range 0.1–0.5 µg/ml of epinephrine, whereas, the responses to ACh are little modified over this range. That is, enhancement of first phase insulin release by epinephrine priming with glucose as the insulinogogue is more dependent on a high epinephrine preincubation concentration than is the enhancement of the first phase of ACh-induced insulin release. Conversely, the first phase of glucose-induced release is more likely to be inhibited by high epinephrine concentrations. The combination of these two effects, particularly on the first phase of insulin release, are such as to suggest that ACh stimulation can more readily ensure appropriate first phase insulin release than can glucose in the face of variation in the intensity of adrenergic stimulation.

To summarize the above, effective mechanisms exist, whereby appropriately timed adrenergic and cholinergic impulses can modify the quantitative and qualitative patterns of insulin release. Further, they indicate that there is temporally related cooperativity between the two systems; that is, the systems can function other than in a simple antagonist fashion. The studies suggest that optimal biphasic responses, and in particular, optimal acute insulin release would be expected when adrenergic stimulation or "tone" to B cells increases during interprandial periods and decreases with onset of insulin release which, in turn is stimulated by both an increase in effective acetylcholine concentration at B-cell receptor sites (through reflex mechanisms, cephalic [45, 46] and local [47, 48] and increases in substrate (glucose) concentration. The availability of such an autonomic mechanism for producing optimal acute insulin responses assumes importance in the light of data which indicates that the acute insulin response to glucose challenge is a prime determinant of the rate of glucose disappearance under physiological conditions (49–53).

Finally, the above studies provide support for the suggestion that the anomalies observed in insulin release in some diabetics (54–56) could be related to anomalous or absent autonomic modification of B-cell function (31, 42, 57). The anomalies include: a quantitative reduction in the amount of insulin released in response to glucose challenge (54) in some, but not all, (58) diabetics with retention of apparently normal responsiveness to theophyllin (59) and glucagon (60), and a "delay" in insulin release in response to glucose challenge (54–56, 61). This latter anomaly perhaps representing the in vivo equivalent (61) of a markedly impaired first phase response to the artificial situation of square wave glucose challenge seen in vitro. That is, deficiency in either epinephrine priming and(or) cholinergic stimulation or anomalous temporal initiation of these effects (with loss of cooperatively) would all tend to reduce the amount of insulin released over both phases, and in particular, would produce relatively greater impairment of the first phase response.

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