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Effects of Prior 5-Hydroxytryptamine Exposure on Rat Islet Insulin Secretory and Phospholipase C Responses

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Glucose-induced insulin secretion is inhibited by 5-hydroxytryptamine (5HT). In the present studies the specificity of 5HT inhibition of release and the potential biochemical mechanisms involved were investigated. Dose-dependent inhibition of 15 m*M* glucose-induced secretion was induced by a prior 3 h incubation with 5HT. At the highest 5HT concentration (500 µ*M*) employed, both first and second phase responses to 15 m*M* glucose were reduced 50–60%. In addition, this level (500 µ*M*) of 5HT virtually abolished 10 m*M* glucose-induced secretion. In contrast, secretion in response to the protein kinase C activator phorbol 12-myristate 13-acetate (500 n*M*) was immune to 500 µ*M* 5HT pretreatment. Glucose usage rates were comparable in both control and 500 µ*M* 5HT-pretreated islets. However, the generation of inositol phosphates and the efflux of ^{3}H-inositol from ^{3}H-inositol-prelabeled islets in response to stimulatory glucose were impaired in parallel with insulin secretion. Based on these observations the following conclusions were reached: (1) 5HT impairs glucose-induced insulin release by altering glucose-induced activation of phospholipase C. (2) Biochemical events distal to phospholipase C remain intact despite this proximal biochemical lesion. (3) Amperometric analysis of 5HT release from 5HT-pretreated islets must take into consideration its profound adverse impact on glucose-induced insulin secretion.

Key Words: Islets; secretion; time-dependent suppression; second messengers; phospholipase C; desensitization.

Introduction

The regulation of insulin secretion from the pancreatic β-cell is a complex phenomenon dependent on a large number of integrated metabolic, ionic, and biochemical events *(1–4)*. In addition to actual measurements of insulin released during agonist stimulation, a number of surrogate markers have been employed to analyze β-cell secretory responses. These include connecting (C)-peptide release *(5,6)* and the efflux of 5-hydroxytryptamine (5HT) from 5HT-preloaded islets. Amperometric studies using 5HT have been used to support the concept that insulin stimulates its own secretion *(7)*. However, the precise physiologic significance of these amperometric studies is not clear because several previous reports, including studies in humans *(8)*, have demonstrated that 5HT receptor activation inhibits secretion from β-cells *(9–12)*. Our interest in elucidating the factors that regulate stimulus–response coupling in the β-cell and the role of phospholipase C (PLC) activation in this process prompted additional studies using islets treated with 5HT.

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Results

Glucose-Induced Release

In the initial series of studies, islets were perifused and stimulated with either 10 m*M* or 15 m*M* glucose after a 3 h incubation with 500 µ*M* 5HT. Control islets incubated with 5 m*M* glucose alone responded to 10 m*M* glucose stimulation with a biphasic insulin secretory response (Fig. 1, top). Peak first and second phase release from these islets averaged 100 ± 31 and 216 ± 42 (n = 6) pg/islet/min, respectively. The capacity of 10 m*M* glucose to stimulate secretion was markedly impaired by prior exposure to 500 µ*M* 5HT. Peak first and second phase release from these islets averaged 27 ± 7 and 35 ± 4 (n = 6) pg/islet/min, respectively. Taking into consideration prestimulatory release rates of 21 ± 7 pg/islet/min, both phases of secretion were reduced more than 90% by 500 µ*M* 5HT.

Additional studies were conducted using islets stimulated with 15 m*M* glucose after exposure to 50–500 µ*M* 5HT. Control islets incubated with 5 m*M* glucose alone for 3 h responded during the subsequent perifusion with a biphasic insulin secretory response (Fig. 1, bottom). Peak first phase release rates averaged 141 ± 16 pg/islet/min (n = 6), and this increased to over 450 pg/islet/min 30 min after the onset of stimulation. When compared to prestimulatory insulin secretion rates of approx 20 pg/islet/min, a 20–25-fold increase in second phase release occurred in response to this glucose concentration.

Incubation for 3 h in the additional presence of 50 µ*M* 5HT was without any deleterious effect on 15 m*M* glucose-induced secretion (results not shown). However, prior in-

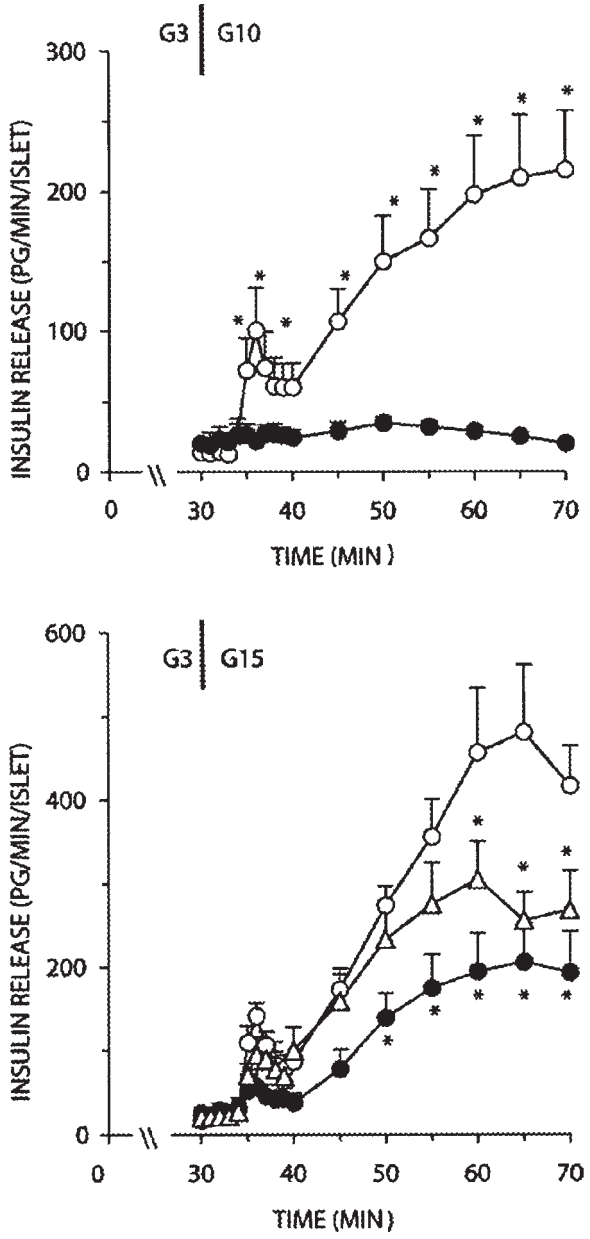

Fig. 1. Insulin secretion from 5HT-pretreated islets. Groups of isolated islets were incubated for 3 h in a 5 m*M* glucose-containing medium plus or minus 5HT. After washing with 5 mL of fresh medium, the islets were perifused for 30 min with 3 m*M* glucose (G3) to establish to basal, stable release rates. **Top.** Control islets (open circles, incubated for 3 h with 5 m*M* glucose alone) or islets pretreated with 500 μ*M* 5HT (closed circles) were perifused for an additional 40 min with 10 m*M* glucose (G10) as indicated by the vertical line. **Bottom.** Control islets (open circles, incubated for 3 h with 5 m*M* glucose alone) or islets pretreated with 200 μ*M* 5HT (open triangles) or 500 μ*M* 5HT (closed circles) were perifused for an additional 40 min with 15 m*M* glucose (G15). Mean values ± SEs for at least 3 experiments are depicted. The asterisk indicates a significant difference at this time point between control and 5HT-pretreated islets. This and subsequent figures have not been corrected for the dead space in the perifusion apparatus, 2.5 mL or 2.5 min with a flow rate of 1 mL/min.

cubation with 200 μ*M* 5HT reduced the sustained insulin secretory response measured 35–40 min after the onset of stimulation by about 35% when compared to the responses of control islets (417 ± 49 vs 269 ± 47 pg/islet/min from controls and 5HT-treated islets, respectively). An even more pronounced inhibitory effect was noted after 500 μ*M* 5HT incubation. In this case, sustained release rates measured 35–40 min after the onset of stimulation now averaged only 194 ± 50 (n = 5) pg/islet/min (Fig. 1, bottom), a decline of more than 50% when compared to release rates from control, non-5HT-treated islets. This level (500 μ*M*) of 5HT was used in all subsequent experiments.

Glucose Metabolism in 5HT-Pretreated Islets

Despite its marked inhibitory effect on 15 m*M* glucose-induced insulin release, incubation of islets for 3 h in 500 μ*M* 5HT had no adverse impact on glucose usage rates measured during a subsequent 1 h incubation. While control islets previously incubated with 5 m*M* glucose used 15 m*M* glucose at rates of 145.9 ± 8.3 pmol/islet/h (n = 5), comparable values in 5HT-pretreated islets averaged 149.4 ± 17.4 pmol/islet/h (n = 4).

Phorbol Ester-Stimulated Insulin Secretion

The specificity of the secretory lesion produced by the 3 h incubation with 500 μ*M* 5HT was investigated next. For these studies, islets were incubated in the presence or absence of 500 μ*M* 5HT. Groups of islets were then loaded into perifusion chambers for secretory studies. After a 30 min stabilization period with 3 m*M* glucose, islets were stimulated with 500 n*M* of the protein kinase C activator phorbol 12-myristate 13-acetate (PMA). As shown in Fig. 2, prior exposure to 500 μ*M* 5HT had no adverse impact on islet secretory response to PMA.

PLC Activation in 5HT-Pretreated Islets

In response to glucose stimulation, the activation of PLC parallels the insulin secretory response *(13–15)*. Moreover, prior sustained exposure of islets to a number of compounds including high glucose impairs both PLC activation and insulin secretion in a parallel fashion *(16–19)*. Two additional studies were conducted to determine the impact of prior 5HT treatment on the activation of PLC. In the first series of studies islets were incubated with ^{3}H-inositol to label their phosphoinositide pools. Also included during the 3 h incubation was 500 μ*M* 5HT. In response to subsequent stimulation with 10 m*M* glucose, control islets responded with a biphasic 5 insulin secretory response (Fig. 3, top). Islets exposed to 5HT during the labeling period were immune to stimulation with this hexose level. The efflux of ^{3}H-inositol in response to 10 m*M* glucose stimulation paralleled the islet insulin secretory responses from control and 5HT pretreated islets. A four- to fivefold increase in ^{3}H-inositol efflux was noted from control islets while this response was abolished after 5HT treatment (Fig. 3, bottom).

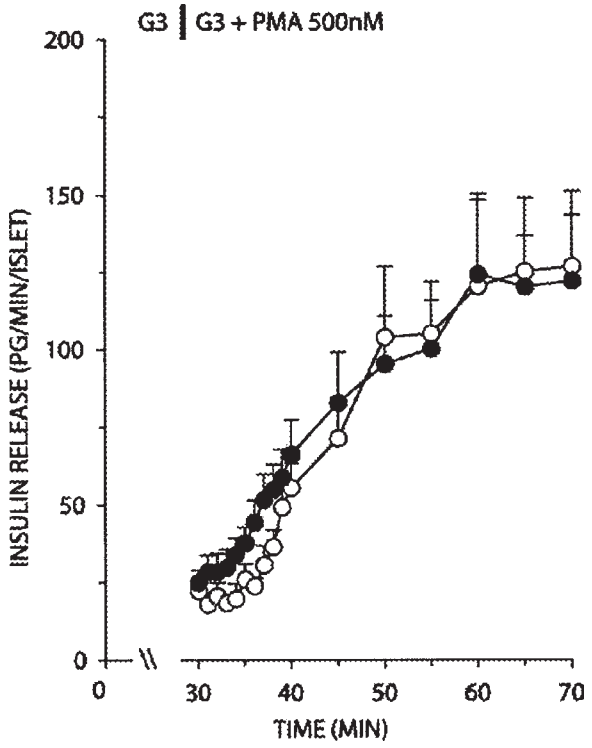

Fig. 2. Phorbol ester–induced secretion from control and 5HT-pretreated islets. Groups of islets were perifused after a 3 h incubation period in 5 m*M* glucose plus or minus 500 μ*M* 5HT. After a 30 min stabilization period with 3 m*M* glucose alone, control islets (open circles) and 5HT-pretreated islets (closed circles) were perifused for an additional 40 min in a medium supplemented with 500 n*M* phorbol 12-myristate 13-acetate (PMA). Mean values ± SEs for at least three experiments are depicted.

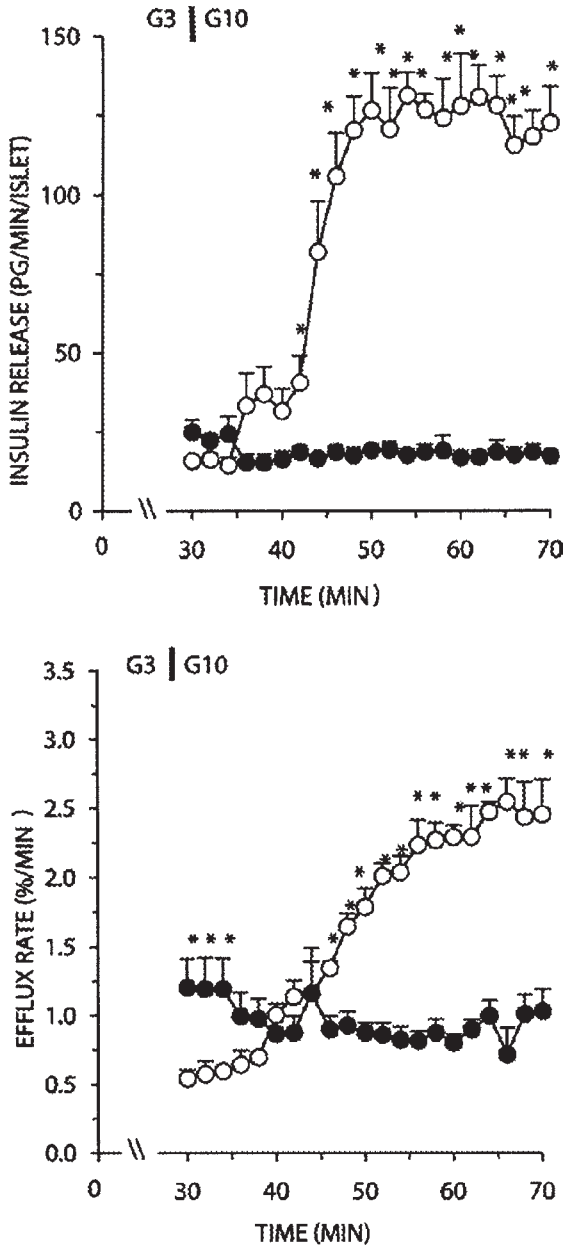

Fig. 3. Insulin secretion and phospholipase C activation in islets. Groups of islets were incubated for 3 h in 5 m*M* glucose containing medium plus or minus 500 μ*M* 5HT. Also included during this time was myo-2-^{3}H-inositol used to label phosphoinositide pools. After washing to remove unincorporated label, both groups of islets were perifused for 30 min with 3 m*M* glucose (G3) and then stimulated for an additional 40 min with 10 m*M* glucose plus 5 m*M* nonradioactive inositol to prevent label reuptake. Insulin secretory rates (**top**) and fractional rates of myo-2-^{3}H-inositol efflux (**bottom**) were monitored in control (open circles, $n = 4$) and 5HT-pretreated (closed circles, $n = 6$) islets. The asterisk indicates a significant difference at this time point between control and 5HT-pretreated islets.

A more direct assessment of PLC activation was obtained by measuring the accumulation of labeled inositol phosphates in response to glucose stimulation. After a 3 h incubation, both control and 5HT-pretreated islets exhibited similar levels of labeled inositol phosphates in the presence of 3 m*M* glucose. Control islets responded to stimulation with 10 or 15 m*M* glucose with significant increments in inositol phosphate (IP) levels. However, and in agreement with the efflux studies above, prior exposure to 5HT significantly reduced inositol phosphate (IP) accumulation in response to the same hexose levels (Table 1).

Discussion

A number of approaches have been utilized to monitor the secretion of insulin from the pancreatic β-cell. Direct measurements of insulin secretion are most numerous but under certain conditions C-peptide measurements *(6,20, 21)* have been also employed. Most recently, amperometric studies measuring the efflux of 5HT from 5HT-preloaded β-cells have also been used as a surrogate marker for insulin secretion *(7)*. However, because 5HT treatment impairs islet responses to glucose, results utilizing this approach have to be cautiously interpreted. Our interest in identifying the factors that regulate insulin secretion prompted further biochemical analysis of the events that may underlie the inhibitory

Table 1
Inositol Phosphate (IP)
Responses of Rat Islets after 5HT Treatment[a]

	IP accumulation (cpm/40 islets/30 min)	
	Controls	5HT pretreatment
1. G3	3,036 ± 246 (n = 7)	3,165 ± 489 (n = 6)
2. G10	10,454 ± 672 (n = 5)	5,231 ± 361* (n = 6)
3. G15	13,137 ± 1,168 (n = 9)	7,730 ± 760* (n = 9)

[a]Groups of islets were incubated in the presence or absence of 500 μ*M* 5HT for 3 h to label their phosphoinositide pools as described in the Methods section. After washing to remove unincorporated label, they were then incubated for 30 min with 3 m*M* (G3), 10 m*M* (G10), or 15 m*M* (G15) glucose. Mean values ± SEs are given and the number of experiments performed is indicated in the parentheses. Asterisk indicates a significant difference between control and 5HT-pretreated islets.

impact of 5HT on stimulated secretion and further comment on our findings follow.

We first confirmed that the concentration of 5HT used to monitor insulin release in amperometric studies is a potent inhibitor of glucose-induced insulin secretion *(10–12)*. In the case of islets stimulated with 10 m*M* glucose, release in response to the hexose was virtually abolished. Higher levels of glucose did result in an increased secretory response from 5HT-pretreated islets but the magnitude of the response achieved was significantly reduced when compared to control islet responses.

Considering the importance of glucose metabolism in determining the stimulatory efficacy of glucose on the secretory process *(22,23)*, we determined if 5HT pretreatment interferes with this biochemical response. Our studies demonstrate that control and 5HT-pretreated islets both metabolized glucose at comparable rates, thus eliminating any obvious defect in glucose usage as the underlying cause of the secretory impairment.

In an attempt to gain further insight into the lesion produced by 5HT, additional experiments were employed to determine if other agonists of insulin secretion were similarly affected. In contrast to its marked inhibitory impact on glucose-stimulated secretion, β-cell responses to the PKC activator PMA were immune to the adverse effect of 5HT. Under conditions where release to an approximately equipotent glucose stimulus was abolished, responses to this pharmacologic activator were unchanged. This finding suggests that the biochemical lesion induced by 5HT is confined to more proximal events in signal transduction cascade.

In a series of previous studies we reported, in agreement with other investigators *(13,14,24,25)*, that events associated with the activation of PLC are important physiologic determinants of glucose-induced secretion *(19)*. In addition, impaired signaling via the PLC cascade results in a reduction in insulin secretion as well. For example, chronic exposure to high glucose, cholecystokinin, carbachol, glucosamine, monomethylsuccinate, or forskolin *(17,26–29)* impair both stimulated insulin secretion and PLC activation in a parallel fashion. Studies reported here reveal that this pathway is also adversely affected after 5HT pretreatment as well and that this lesion parallels the defect in secretion. Both the efflux of ^{3}H-inositol and the accumulation of ^{3}H-inositol phosphates in response to glucose stimulation are significantly reduced after 5HT exposure.

Precisely how the activation of PLC is desensitized by 5HT pretreatment remains to be established and further studies will be necessary to address this issue. This task is, however, complicated by the existence of multiple receptor types for the amine, the differential activation events of 5HT on target tissues including the activation PLC and phospholipase A_2, and the heterologous interactions that occur between this receptor type and others *(30,31)*. Moreover, a large number of potential mediators are also generated by the activation of 5HT receptors *(31)* including a number of arachidonic acid–derived compounds with established inhibitory effects on islet cell responses *(32,33)*. Despite the uncertainties regarding its mechanism of action, it is clear that 5HT exposure results in a parallel reduction of both PLC activation and insulin secretion. This lends credence to the concept that PLC plays a particularly important role in stimulus-response coupling in both human and rodent islets *(8,10–12)*.

Materials and Methods

The detailed methodologies employed to assess insulin output from collagenase-isolated islets have been described previously *(34,35)*. Male Sprague–Dawley rats (weighing 300–475 g) were purchased from Charles River and used in all studies. All animals were treated in a manner that complied with the NIH Guidelines for the Care and Use of Laboratory Animals. The animals were fed *ad libitum*. After Nembutal- (pentobarbital sodium, 50 mg/kg; Abbott, North Chicago, IL) induced anesthesia, islets were isolated by collagenase digestion and handpicked, using a glass loop pipet, under a stereomicroscope into Krebs–Ringer Bicarbonate (KRB) supplemented with 3 m*M* glucose. They were free of exocrine contamination.

Perifusion Studies

Islets were loaded onto Nylon filters (Sefar America Inc., Kansas City, MO) and incubated for 3 h in 240 μL of KRB supplemented with 5 m*M* glucose plus or minus 50–500 μ*M* 5HT. After the incubation the islets were perifused in a KRB buffer at a flow rate of 1 mL/min for 30 min in the presence of 3 m*M* glucose to establish basal and stable insulin secretory rates. After this 30 min stabilization period, they were then perifused with the appropriate agonists as indicated in

the figure legends and the Results section. Perifusate solutions were gassed with 95% O_2/5% CO_2 and maintained at 37°C. Insulin released into the medium was measured by radioimmunoassay *(36)*.

Islet Labeling for IP and Myo-2-^{3}H-Inositol Efflux Studies

Groups of 18–26 islets were loaded onto nylon filters and incubated for 3 h in a myo-2-^{3}H-inositol–containing KRB solution made up as follows: 10 μCi of myo-2-^{3}H-inositol (specific activity 16–23 Ci/mmol) were placed in a 10 mm × 75 mm culture tube. To this aliquot of tracer, 255 μL of warmed (to 37°C) and oxygenated KRB medium supplemented with 5.0 m*M* glucose plus or minus 500 μ*M* 5HT were added. After mixing, 240 μL of this solution was gently added to the vial with islets. The vial was capped with a rubber stopper, gassed for 10 s with 95% O2/5% CO_2, and placed in a metabolic shaker at 37°C. After 90 min the vials were again gently oxygenated. After the labeling period, the islets were washed with 5 mL of fresh KRB and used for IP measurements (see below) or to assess the efflux of myo-2-^{3}H-inositol.

The efflux of myo-2-^{3} H-inositol from prelabeled islets was assessed during a dynamic perifusion. This technique allows the parallel assessment of both insulin secretion and phosphoinositide hydrolysis from the same perifusate samples *(15,24,25)*. After the 3 h labeling period in the presence or absence of 500 μ*M* 5HT, the islets were perifused with 3 m*M* glucose for 30 min and for an additional 40 min with 10 m*M* glucose plus 5 m*M* nonradioactive inositol. Fractional efflux rates of myo-2-^{3}H-inositol were calculated as previously described *(37)*.

Labeled inositol phosphates in islets were determined as follows. After the 3 h labeling period islets were washed with 5 mL KRB to remove free labeled inositol. The islets on Nylon filters were then placed in small glass vials. Added gently to the vial was 400 μL of warmed (to 37°C) KRB supplemented with 10 m*M* LiCl to prevent IP degradation and the appropriate agonists as indicated. The vials were capped and after 30 min the generation of IPs was stopped by adding 400 μL 20% perchloric acid. Total IPs formed were then measured using Dowex columns as described previously *(38–40)*.

Glucose Utilization Rates

The usage of glucose was measured by determining the rate of ^3H_2O formation from 5-^{3}H-glucose *(22,41)*. After the 3 h incubation in the presence or absence of 500 μ*M* HT, the islets were incubated in 0.125 mL of 15 m*M* glucose supplemented with tracer 5-^{3}H-glucose. The ^3H_2O formed during the subsequent 1 h incubation was separated from the unused ^{3}H-glucose as described previously *(22)*.

Reagents

Hank's solution was used for the islet isolation. The perifusion medium consisted of 115 m*M* NaCl, 5 m*M* KCl, 2.2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 24 m*M* $NaHCO_3$, and 0.17 g/dL bovine serum albumin. The ^{125}I-labeled insulin for the insulin assay, 5-^{3}H-glucose, and the ^3H_2O for the glucose usage studies were purchased from PerkinElmer Life Sciences (Boston, MA). ^{3}H-Inositol was purchased from Amersham (Arlington Hts, IL). Bovine serum albumin (RIA grade), glucose, phorbol 12-myristate 13-acetate (PMA), inositol, and the salts used to make the Hank's solution and perifusion medium were purchased from Sigma (St. Louis, MO). Rat insulin standard (lot #615-ZS-157) was the generous gift of Dr. Gerald Gold, Eli Lilly Co. (Indianapolis, IN). Collagenase (type P) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN).

Statistics

Statistical significance was determined using the Student's *t* test for unpaired data or analysis of variance in conjunction with the Newman-Keuls test for unpaired data. A p value ≤ 0.05 was taken as significant. Values presented in the figures and results represent means ± SEs of at least three observations.

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