Glucagon and Insulin Release

Influence of Drugs Affecting the Autonomic Nervous System

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SUMMARY

Isolated rat islets of Langerhans were used to study the direct effect of autonomic nervous system compounds on immunoreactive glucagon (IRG) and insulin (IRI) release. None of the substances affected IRG release. Epinephrine (10μM) and norepinephrine (10μM) blocked IRI release in response to 150 mg./100 ml. glucose but did not block the effect of 300 mg./100 ml. glucose. Acetylcholine (3 μM) stimulated IRI release but isoproterenol had no effect.

Because the adenylcyclase system may play a role in adrenergic receptor mechanisms, the effects of substances which alter cellular levels of cyclic AMP were also studied. Again, none of the compounds altered IRG levels in the incubation media. Aminophylline (100 μM) stimulated IRI release but dibutyryl cyclic AMP and prostaglandin E_1 were without significant effect.

The results suggested that neither autonomic nervous system compounds or the adenylcyclase system directly affect IRG release. The physiological significance of the direct inhibitory effect of substances with alpha adrenergic properties on IRI release is questioned in view of the very high concentration required for effect. DIABETES *20*:78-82, February, 1971.

Alteration of insulin secretion appears to be an important mechanism for the influence of the autonomic nervous system on carbohydrate metabolism. Alpha adrenergic agents, such as epinephrine[1] and norepinephrine,[2] inhibit insulin secretion while beta adrenergic agents,[3] acetylcholine,[4] and vagal stimulation[5] promote insulin secretion. Because these agents also influence capillary blood flow, isolated islets of Langerhans were used in this study to determine if they directly affect insulin and glucagon secretion. In addition, as adenylcyclase is thought to play a role in adrenergic receptor mechanisms,[6] substances which affect cyclic AMP levels were also studied.

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METHODS

Islets were isolated from 200-300 gm. male Wistar rats by a previously described modification[7] of the collagenase method of Lacy and Kostianovsky.[8] After isolation in chilled Hanks solution, ten islets per flask were preincubated for one hour at 37°C. in 1 ml. Krebs Hensleit bicarbonate buffer (KHB) with 60 mg./100 ml. glucose, and thereafter washed once with saline. Then 1 ml. fresh, chilled KHB containing 150 mg./100 ml. glucose was added with or without a test substance. An aliquot of the incubation media was immediately removed for assay of the baseline hormone concentration and the islet flask was gassed with 95 per cent O_2-5 per cent CO_2 for three minutes and incubated in a metabolic shaker at 37°C. for thirty minutes. The islet flasks were chilled and another aliquot was removed for hormone assay. Results were expressed as the (net) hormone released per ten islets per thirty minutes. Trasylol, 1000 KIU/ml., was added to the incubation media to prevent insulin and glucagon degradation.[7] Control and test incubation vessels were alternated consecutively in each experiment. The procedure for the epinephrine timed release experiment is described under *Results*.

Double antibody radioimmunoassay technics were used to measure insulin (IRI)[9] and glucagon (IRG)[10] in the incubation media. Control and test media were always run in the same assay.

RESULTS

Epinephrine. Epinephrine bitartrate had no effect on immunoreactive glucagon (IRG) release but resulted in a 50 per cent inhibition of immunoreactive insulin (IRI) release at a minimum concentration of 10 μM

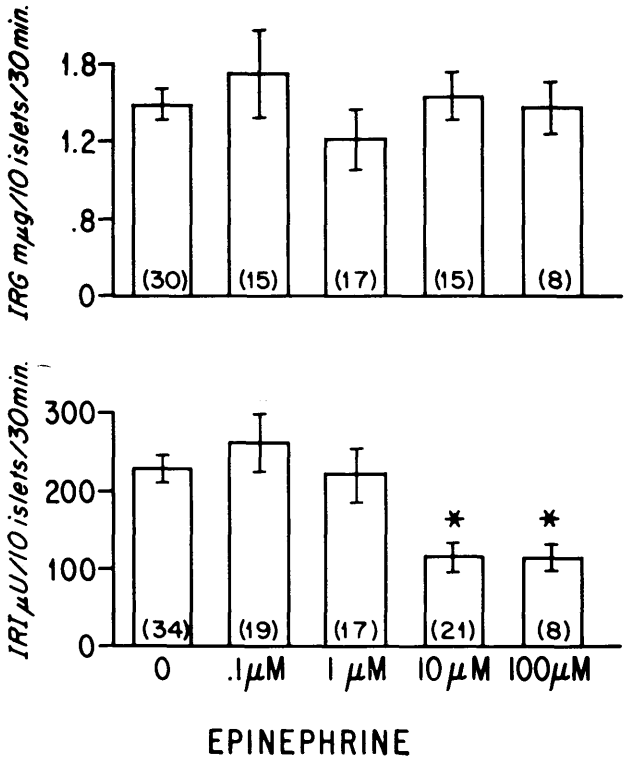

FIG. 1. The effect of epinephrine on immunoreactive glucagon and insulin release. Results expressed as mean ± S.E.M. hormone released per 10 islets per 30 minutes. Number of experiments indicated at the base of each bar. Asterisk signifies $p<.05$.

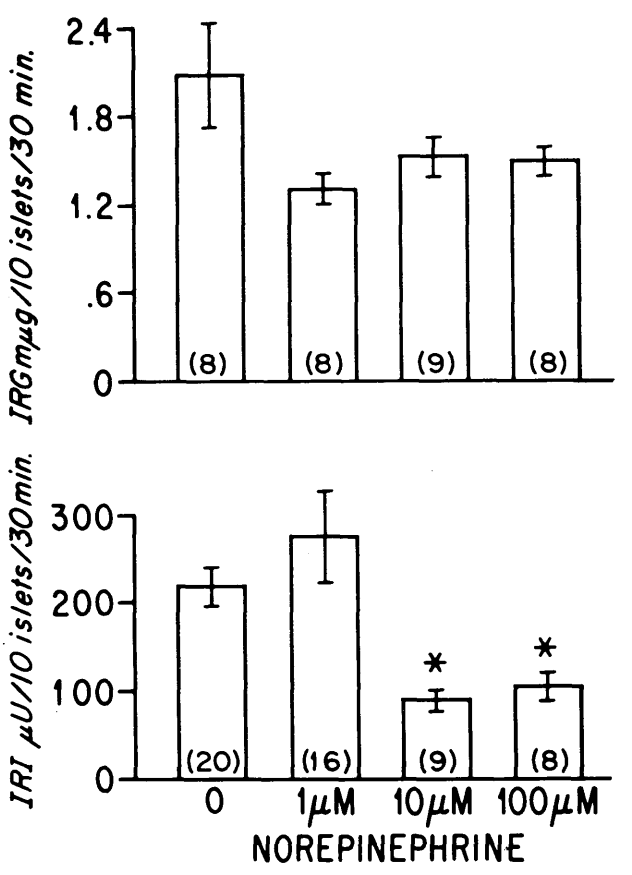

FIG. 2. The effect of norepinephrine on immunoreactive glucagon and insulin release expressed as mean ± S.E.M. hormone released per 10 islets per 30 minutes. Number of experiments indicated at the base of each bar. Asterisk signifies $p<.05$.

(figure 1). Increasing the epinephrine concentration to 100 μM caused no further depression of insulin release.

Norepinephrine. IRG release during incubation with norepinephrine (figure 2) was no different than that in the controls. IRI release was inhibited in a manner similar to that observed with epinephrine with a maximum decrease of 59 per cent achieved with 10 μM norepinephrine.

Isoproterenol. Isoproterenol, a substance with predominantly beta adrenergic activity, had no significant effect on IRG or IRI release (figure 3).

Acetylcholine. The release of IRG was not altered by the addition of acetylcholine (3 μM) to the incubation media (figure 4). The same concentration, however, increased IRI release by 51 per cent compared with the control flasks.

Agents Affecting Cellular Levels of Cyclic AMP. Table 1 summarizes the results of incubating islets with agents known to affect the adenylcyclase system. None of the substances tested altered IRG release. Islets incubated with dibutyryl cyclic AMP showed a wide variability in response. Aminophylline caused a 245 per cent increase in IRI release when added in a concentration of 1000 μM. Prostaglandin E_1, which inhibits adenylcyclase in certain tissues,[11] had no significant effect on IRG or IRI release.

Timed Release with Epinephrine. To study the effect of the addition of epinephrine on glucose-stimulated insulin release, the following experiment was performed (figure 5). Islets were isolated and preincubated in KHB with 60 mg./100 ml. glucose as described in *Methods.* After washing with saline, 1 ml. of chilled KHB with 300 mg./100 ml. glucose was added to each incubation vessel containing ten islets. After gassing, the vessels were incubated at 37 degrees in a metabolic shaker. Eight minutes after starting the incubation, an aliquot (0.02 ml.) was removed for baseline IRI measurement. Eight minutes later another aliquot was removed and epinephrine (0.02 ml.) was added to alternate vessels to make a final concentration of 100 μM. Alternate control vessels received normal saline (0.02

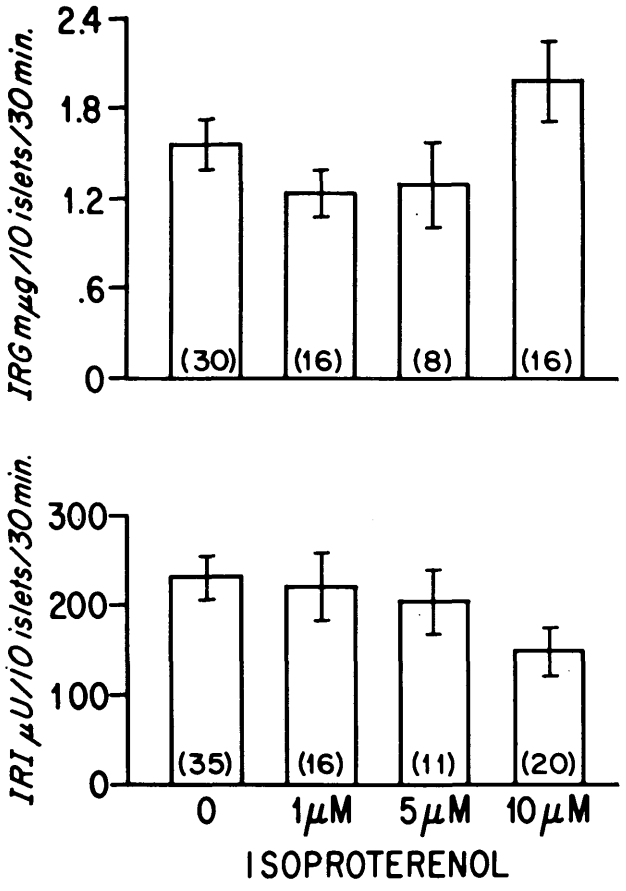

FIG. 3. Effect of isoproterenol on immunoreactive glucagon and insulin release expressed as mean ± S.E.M. hormone released per 10 islets per 30 minutes. Number of experiments indicated at the base of each bar. Isoproterenol had no significant effect on the release of either hormone.

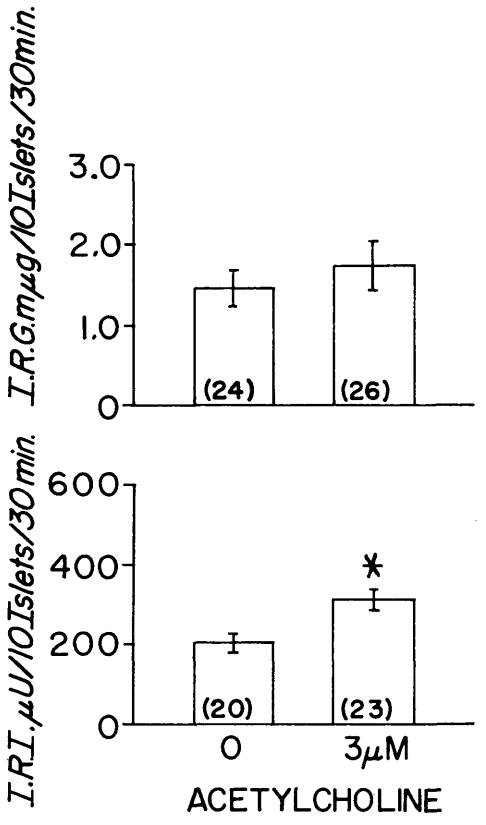

FIG. 4. Effect of acetylcholine on immunoreactive glucagon and insulin release expressed as mean ± S.E.M. per 10 islets per 30 minutes. Number of experiments indicated at the base of each bar. Asterisk signifies $p<.05$.

ml.). Additional aliquots were removed every eight minutes for IRI assay for a total incubation period of forty minutes. As depicted in figure 5, the addition of epinephrine did not significantly affect IRI release stimulated by 300 mg./100 ml. glucose.

DISCUSSION

Insulin and glucagon release mechanisms in isolated mammalian islets have been shown to respond to some of the stimuli and manipulations known to affect islet cell function *in vivo,* including glucose[7,8,12] and other monosaccharides,[7,13,14] pentitols,[15] starvation,[16] tolbutamide,[13,14,17] diazoxide,[17] glucagon,[13,14,17] epinephrine,[14,25] and theophylline.[15] Thus, the isolated islet technic seems to be an excellent system for studying directly the functional activity of the endocrine pancreas. The failure of isolated islets to respond to certain stimuli, and some of the problems in the use of this system, have been discussed.

With use of this technic, the present studies showed that glucagon release is not affected by alpha or beta adrenergic agents, by acetylcholine, or by agents which alter cyclic AMP levels. It had been postulated that the central nervous system might enhance the secretion of glucagon, presumably via the autonomic nervous system.[18] The recent demonstration of adrenergic and cholinergic innervation of the mammalian pancreas[19] further suggested that the autonomic nervous system may help regulate glucagon release. However, the present studies indicate that the chemical mediators of the autonomic nervous system do not directly affect glucagon release, observations recently confirmed by Chesney and Scholfield.[20] In contrast to our findings, however, these same authors observed a potent glucagon-releasing effect by theophylline when incubated with isolated islets in the presence of 60 mg./100 ml. glucose.[20] We observed no effect with aminophylline in the presence of 150 mg./100 ml. glucose.

The inhibition of insulin release by epinephrine and

TABLE 1

Additions to Incubation Buffer	IRG Release mμg./10 Islets/30 min.	No. Exper.	IRI Release μμg./10 Islets/30 min.	No. Exper.
D-CAMP 125 μg./ml.	1.266 ± .143	19	255 ± 60	13
Control	1.422 ± .225	13	117 ± 39	11
Aminophylline 10μM	1.073 ± .129	8	207 ± 35	8
Control	1.073 ± .154	6	163 ± 17	6
Aminophylline 1000μM	2.207 ± .425	12	404 ± 81*	15
Control	1.601 ± .200	15	165 ± 37	11
Prostaglandin E_1, 1μg./ml.	1.357 ± .159	8	225 ± 37	8
Control	1.070 ± .155	6	163 ± 17	6

*$p<.05$
D-CAMP = dibutyryl cyclic AMP

norepinephrine is qualitatively similar to the results in other in vitro studies using the pancreas slice technic[4,21,22] and the isolated islet system.[14,15] In all of these reported studies an inhibition of insulin secretion was observed with approximately 1 μM epinephrine (200/mug./ml.). We found that a minimum concentration of 10 μM was required to impair insulin release, that 1 μM did not have an effect. Nevertheless, even a concentration of 1 μM is at least 200 times the reported plasma concentration of epinephrine.[23] When used in high concentration *in vivo,* epinephrine causes beta cell damage.[24] The impairment of insulin secretion obtained with infusion of physiological concentrations of epinephrine *in vivo*[1] may result in part from an indirect effect on beta cell function. One such indirect mechanism may involve an impairment of the microcirculation of the islet. Epinephrine, in low concentration, has been shown to markedly impede islet blood flow.[25,26,27] The functional significance of the observed impairment of insulin secretion in vitro by pharmacological concentrations of catecholamines is questionable. As shown in figure 5, with intense stimulation of insulin release by glucose, the addition of a high concentration of epinephrine caused little effect during the relatively short period of observation.

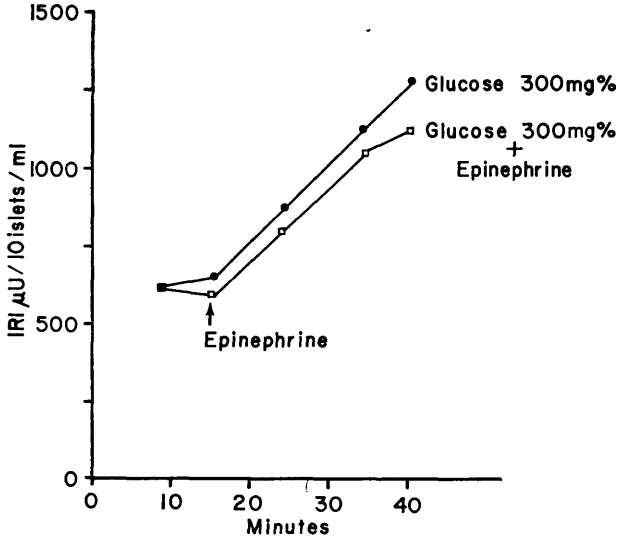

FIG. 5. The effect of epinephrine on insulin release from islets incubated in 300 mg./100 ml. glucose. Epinephrine was added to experimental flasks after 8 minutes and an equal volume of 0.9 per cent NaCl was added to control flasks. There was no significant difference between the two groups. Each point represents the mean of 6 or 7 islet incubation experiments.

Increased insulin release in response to aminophylline was demonstrated and confirms similar results by others using theophylline.[28] It is not certain if the aminophylline effect is due entirely to inhibition of phosphodiesterase with accumulation of intracellular cyclic AMP levels. The direct insulin releasing property of cyclic AMP was not demonstrable in our experiments, possibly because of the wide variability of response. Malaisse, et al.[28] demonstrated stimulation of insulin release from pancreas slices by isopropylnorepinephrine by simultaneous incubation with phenoxybenzamine, an alpha adrenergic blocking agent. We observed no effect of isopropylnorepinephrine on insulin release from isolated islets. Acetylcholine augmented insulin release as previously demonstrated by other in vitro studies.[4,21]

Changes in insulin release in response to all of the agents tested in this study were observed without simultaneous changes of glucagon concentration in the media. Therefore, if pancreatic glucagon plays a role in stimulating insulin secretion, it does so without reflecting changes in glucagon concentration outside of the islet.

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