Insulin Release
Demonstration of a Priming Effect of 3-Isobutyl-1-Methyl-Xanthine (IBMX) on Islets of Langerhans
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SUMMARY
Exposure of isolated rat pancreatic islets to 1 mM 3-isobutyl-1-methyl-xanthine (IBMX) resulted in a 5.7-fold increase in the rate of insulin release. After a 30-min rest period under basal conditions, reexposure of the islets to 1 mM IBMX resulted in an enhanced rate of release when compared with either a timed control— islets simultaneously exposed to IBMX for the first time—or with the first priming response to IBMX. The possibility of a continuous priming effect was suggested by the gradually rising rate of insulin release during exposure to IBMX. It is concluded that islets have a memory for IBMX exposure that results in increased responsiveness to subsequent stimulation by IBMX. DIABETES 30:754-756, September 1981.

Exposure of pancreatic islets to high stimulatory concentrations of glucose has a priming effect on the ß-cells such that a subsequent response to a glucose stimulus gives rise to an enhanced rate of insulin secretion. The ß-cell is said to have a "memory" for previous glucose exposure. After priming, the responses to other secretagogues, as well as glucose, are enhanced. Glucose priming enhances the subsequent responses to agents as varied as leucine, tolbutamide, barium, and 3-isobutyl-1-methyl-xanthine (IBMX). The mechanism of this priming effect is not understood but is currently thought to be a property of glucose addition to the ß-cell and not a property of other secretagogues, except for glucose metabolites such as glycerol dehyde. It is reported that IBMX, for example, does not have the ability to prime islets so that they do not give enhanced responses to subsequent stimulations by IBMX or glucose. In view of the need to understand the mechanism of priming, we have performed experiments with IBMX under somewhat different conditions from those previously reported. It was found, contrary to the published literature, that IBMX has a marked priming effect and that islets do have a memory for previous exposure to IBMX.

METHODS
Pancreatic islets were isolated by the collagenase digestion technique from male Wistar rats weighing 150 g. The islets were perifused using batches of 20 islets per 70-µl chamber as described previously. The perifusate consisted of Krebs-Ringer bicarbonate (KRB) buffer containing 2.5 mM CaCl₂, 0.2% bovine serum albumin, and 2.8 mM glucose. The flow rate was 1.0 ml/min. From time zero to 48 min the islets were perifused under basal conditions (KRB buffer and 2.8 mM glucose). At 48 min one set of the islets was exposed to KRB buffer, 2.8 mM glucose, and 1 mM IBMX (experimental) while another set of islets remained under basal perifusion conditions. Forty-five minutes later, at time 93 min, the experimental islets were returned to basal conditions. After a further 30 min under basal conditions, both sets of islets were subjected to KRB, 2.8 mM glucose, and 1 mM IBMX. These stimulatory conditions were maintained for a further 42 min when, at time 165 min, the experiments were terminated. Thus it is possible to evaluate the effect of a previous stimulation by IBMX on a subsequent response of the islets.

The islet perifusates were not collected during the first 40-min (equilibration) period. Thereafter, samples were collected at 1-min intervals during the first few minutes after the start or cessation of a stimulation period, and at 5-min intervals for the remainder of the stimulation periods, when insulin was being released at fairly constant rates. Aliquots of each collected sample were assayed in duplicate for immunoreactive insulin content by the charcoal separation technique of Herbert et al. using rat insulin standard. Guinea pig anti-pork insulin serum was generously provided by Dr. P. Wright.
RESULTS
The results of the experiments are shown in Figure 1. The first part of the experiment consisted of treating the experimental islets with 1 mM IBMX and leaving the controls under basal conditions. Basal insulin release was constant throughout the first 2 h at between 5 and 8 pg/islet/min. When 1 mM IBMX was added to the experimental islets at 48 min, a prompt rise in the rate of insulin release occurred. The stimulation was characterized by a sevenfold increase in release to a peak rate 3–5 min after exposure to the IBMX (i.e., at 51–53 min). After the peak, the rate of insulin release fell to a nadir at 56 min and then increased again. Insulin release at the nadir was significantly lower than at subsequent time points (e.g., P < 0.01 at 60 min and <0.02 at 90 min). Removal of the IBMX from the perifusate allowed a rapid return of the elevated rate of insulin release to control rates.

A similar pattern of response was observed in the control islets when they were exposed to 1 mM IBMX at 123 min. The peak rate, which occurred 3 min after IBMX exposure, was followed by a nadir, and then again by significantly increased release (e.g., P < 0.02 at 140 and 165 min).

The second part of the experiment compared the response to 1 mM IBMX by control islets with the response in the experimental islets that had had prior exposure to IBMX. It is easily apparent from Figure 1 that the primed islets responded to IBMX with a greater rate of insulin release than the control islets. Peak values for the control islets were 75 pg/islet/min and for the experimental islets 119 pg/islet/min, Δ = 44 ± 13, P < 0.02. The primed islets exhibited significantly increased release rates, when compared with the control islets, from minute 126 (3 min after IBMX exposure) until 160 min when significance was lost. The primed islets also demonstrated a significantly greater response during the second stimulation when compared with their first (priming) stimulation. The peak value of 119 pg/islet/min achieved during the second stimulation was greater than that achieved during the first exposure to IBMX, i.e., 58 pg/islet/min, Δ = 61 ± 13 pg/islet/min, P < 0.01. The release rate was higher from 2 min after exposure to IBMX until 165 min when significance was lost. Interestingly, the primed islets had a different pattern of response in that there was no nadir following the peak. Instead, the insulin release rate fell to a plateau and remained constant thereafter. This differs from unprimed islets, which had increasing rates of insulin release after the nadir. Thus the experimental and control islets approach similar insulin release rates at 160–165 min.

Integrated insulin release for the stimulation periods (48–90 min and 123–165 min, respectively) are shown in Table 1. The primed islets released significantly more insulin than unprimed controls. Also they released more insulin during their second exposure to IBMX (in the primed state) than during the first exposure. There was no significant difference between the amount of insulin released by the experimental islets during their first exposure to IBMX in the unprimed state (48–90 min) and the control islets when exposed to IBMX (123–165 min). The unprimed experimental islets released 1887 ± 291 pg/islet versus 2169 ± 331 pg/islet for the control, Δ = 282 ± 237 pg/islet, P > 0.2.

DISCUSSION
Contrary to previous reports, this study demonstrates that IBMX exerts a priming effect upon pancreatic islets that results in a prompt enhancement of the insulin release response to a subsequent stimulation. Thirty minutes after the end of a 45-min stimulation with IBMX, a second stimulation resulted in a 60% increase in the rate of insulin release relative to the first response. Thus the islets have a memory for

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<td>Total amount of insulin release during comparable stimulation periods in response to 1 mM IBMX. Results are expressed as pg immunoreactive insulin released per islet ± SEM (N = 8)</td>
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<td>48–90 min</td>
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FIGURE 1. Stimulation of insulin release by IBMX. 2.8 mM glucose was present throughout the perifusions of both control and experimental islets. Control islets were exposed to 1 mM IBMX from 123 to 165 min. Experimental islets were exposed from 48 to 93 min and from 123 to 165 min. Insulin release by the experimental islets was significantly greater than the control islets at all time points from 126 to 155 min (126 min, P < 0.02; 127–145, P < 0.01; 150–155, P < 0.03). Similarly, insulin release by the experimental islets during the second exposure to IBMX was significantly greater than during the first exposure at comparable time points (126 vs. 50 min, P < 0.02; 126–150 min vs. 51–75 min, P < 0.01; 155 vs. 80 min, P < 0.05; 160 vs. 85 min, P < 0.02).
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IBMX exposure that lasts at least 30 min. It is interesting that
the response of unprimed islets was qualitatively as well as
quantitatively different from that of the primed islets. The un-
primed islets exhibited a first peak followed by a nadir and a
subsequent increase in insulin release, which suggested a
continuous priming process during exposure to IBMX. This
is similar to the idea that the rising part of glucose-induced
second-phase insulin release is due, in part, to a self-mag-
nifying effect of glucose. In contrast, primed islets did not
exhibit the nadir or secondary rise in insulin release but re-
mained at a plateau of release (in a fully primed state?)
higher than that of unprimed islets until 160 min. At this time
the unprimed islets had increased their insulin release to a
rate similar to that of the primed islets.

The reason for the difference between these results, in
which a priming effect of large magnitude was detected,
and previous studies, in which priming was not detected, is
not clear. However, in studies with perfused pancreas, in
which IBMX priming of a subsequent IBMX response was
not seen, the priming stimulus was applied for only 10 min,\(^7\)
which may be insufficient for priming. In other experiments
the degree of stimulation of insulin release by IBMX was
slight.\(^8\) Similarly, in experiments with isolated islets in which
a priming effect of IBMX toward a subsequent glucose re-
sponse was sought, the initial response to IBMX was only a
70% increase over the baseline (3.3 mM glucose) rate.\(^6\)
Thus the priming signal was weak and one might not expect
to see priming under these conditions. In contrast, a 570%
increase in the rate of insulin release was obtained in the
experiments reported here (see Figure 1).

In attempting to understand the mechanism of priming, it
is important to know whether IBMX priming is the same as
glucose priming. If they prove to be similar, then the loca-
tion of the site and mechanism of priming should be more
easily understood because of the restriction of the mecha-
nism to those sections of stimulus-secretion coupling that
are common to both glucose and IBMX.

ACKNOWLEDGMENT
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