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Effect of 2-Deoxyglucose on [^{32}P] Phosphate and Insulin Release from Perifused Rat Pancreatic Islets

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Summary. The effect of 2-deoxyglucose on glucose mediated insulin and [^{32}P]phosphate release was studied by perifusion of isolated rat pancreatic islets. When islets were perifused with media containing 2.8 mmol/l glucose and 20 mmol/l 2-deoxyglucose for 60 minutes and then exposed to media containing 8.3 or 16.7 mmol/l glucose and 20 mmol/l 2-deoxyglucose for the next 15 minutes, insulin release at either glucose concentration was prompt but blunted. Similarly, islets preincubated (90 min) with [^{32}P] orthophosphate, then perifused with 20 mmol/l 2-deoxyglucose for 75 min and stimulated by either 8.3 or 16.7 mmol/l glucose for the final 15 minutes of 2-deoxyglucose exposure demonstrated obtundation of [^{32}P]phosphate release. Perifusion of islets with 20 mmol/l 2-deoxyglucose alone induced no heightened ^{32}P efflux. These studies suggest that 2-deoxyglucose affects initial events in stimulus-secretion coupling of glucose mediated insulin release.

Key words: Insulin release, pancreatic islets, 2-deoxyglucose, glucose, phosphate efflux.

2-Deoxyglucose (2-DG) inhibits the metabolism of glucose in a variety of tissues by its interference with glucose phosphate isomerase [1] and glucose phosphate dehydrogenase [2]. Although assumed to affect islet glucose metabolism in a similar manner [3, 4], it does not inhibit glucose oxidation in mouse or rat islets [5, 6]. In the rat islet 2-DG inhibits glucose stimulated calcium uptake [7]. During static incubation, perfusion, and perifusion, 2-DG inhibits first and second phase insulin release [4, 7–11].

An index of stimulus-secretion coupling in islets is the rapid transient efflux of orthophosphate with glucose stimulated insulin release [12–14]. We have used this index to study the effects of 2-DG on glucose mediated stimulus-secretion coupling in islets.

Materials and Methods

Animals

Adult female Long Evans rats were purchased from Simonsen Laboratories, Inc. (Gilroy, Calif.). Rats weighed 200–400 g and were fed rat chow (Allied Mills, Inc., Chicago, Illinois) ad libitum.

Experimental Procedures

Islets of Langerhans were isolated by collagenase digestion [15, 16] following pentobarbital anaesthesia. After mincing, pancreata were digested in Hank's solution containing collagenase (type IV, Worthington Biochemical Corp., Freehold, N. J.). When digestion was complete the islets were washed 6 times with Hank's solution containing no phosphate and were then isolated within 1 h with the use of a dissecting microscope and a fine bore siliconized pipette (100–200 islets were obtained from two pancreata).

Islets were placed in perifusion chambers (Millipore plastic filtration units, [Millipore Corp., Bedford, Mass.], 1.0 ml capacity) and perifused with Krebs-Ringer bicarbonate (KRB) solution containing 0.5 mmol/l Na_2HPO_4, as previously described [12]; the dead space of the entire system was 2.5 ml and the flow rate was 1.0 ± 0.1 ml/min.

Islets were prelabelled in with [^{32}P]orthophosphate (New England Nuclear, Boston, Mass.) by suspending 100 islets in modified KRB containing 0.5 mmol/l Na_2HPO_4, to which had been added 150 μCi of carrier free inorganic [^{32}P]phosphate. The suspension was gassed for 5 min with 95% O_2–5% CO_2 and incubated at 37° C for 90 min at 80 oscillations/min under the same gas phase. Following incubation islets were washed 6 times with KRB containing 1.0 mmol/l Na_2HPO_4 and were then placed in the perifusion chambers [12].

For the initial 60 min islets were perifused with KRB containing 1.0 mmol/l Na_2HPO_4 with or without 2-DG. A basal efflux of insulin or [^{32}P]phosphate was obtained from 40 through 60 min and perifusion collection began after 40 min. The "basal period" for insulin release was from 40 through 60 min and for [^{32}P]phosphate from 55 through 60 min. At 60 min the inlet of one or more

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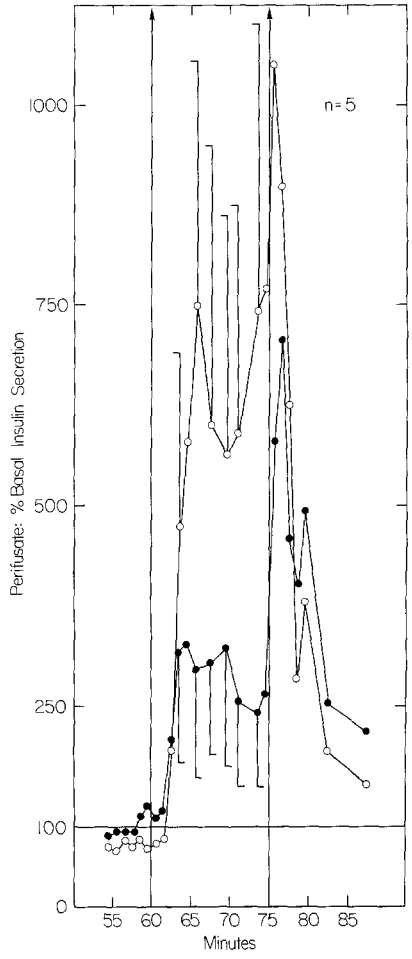

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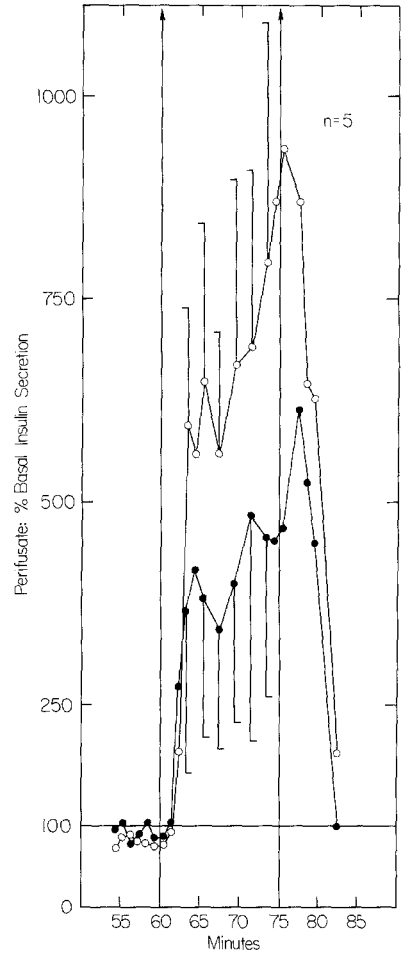

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Fig. 1. Effect of 20 mmol/l 2-DG on insulin release in response to 8.3 mmol/l glucose. Islets in one chamber (○—○) were perifused with KRB-2.8 mmol/l glucose for the first 60 min, then with 8.3 mmol/l glucose from 61–75 min, and finally with 2.8 mmol/l glucose after 75 min. In the second chamber (●—●) islets were perifused with KRB-2.8 mmol/l glucose and 20 mmol/l 2-DG for the first 60 min, then with 8.3 mmol/l glucose and 20 mmol/l 2-DG from 61–75 min, and finally after 75 min with 2.8 mmol/l glucose. Insulin release from the first chamber corresponding to 100% basal was 9.6 ± 0.6 $\mu U \times ml^{-1} \times min^{-1}$ per 25 islets and from the second chamber 14.3 ± 1.0 $ml^{-1} \times min^{-1}$ per 25 islets. Each point represents the mean of separate experiments. Vertical bars indicate SEM

Fig. 2. Effect of 20 mmol/l 2-DG on insulin release in response to 16.7 mmol/l glucose. Experimental design and presentation of results are as in Fig. 1, except that 16.7 mmol/l glucose was used instead of 8.3 mmol/l. Insulin release from the first chamber corresponding to 100% basal was 11.6 ± 1.2 $\mu U \times ml^{-1} \times min^{-1}$ per 25 islets and from the second chamber was 16.4 ± 1.3 $\mu U \times ml^{-1} \times min^{-1}$ per 25 islets. Each point represents the mean of 5 separate experiments. Vertical bars indicate SEM

perifusion channels was transferred with a three way valve (Hamilton Co., Reno, Nev.) to a different KRB solution. In all experiments the "experimental periods" existed from 61 through 75 min.

All reagents used were analytical grade. Glucose was purchased from the National Bureau of Standards (Washington, D. C.) and 2-deoxy-D-glucose from Sigma Chemical Co. (St. Louis, Missouri).

Assays

A double antibody radioimmunoassay was employed to determine total immunologically reactive insulin [17, 18] using rat insulin standards (Novo Research Institute, Bagsvaerd, Denmark), guinea pig antiporcine insulin antiserum (Miles Laboratory, Elkhart, Ind.) and goat anti-guinea pig gamma globulin (Antibodies, Inc., Davis, Calif.) as second antibody. Each sample was assayed in triplicate. Insulin release from perifused islets was expressed as the percent of mean basal insulin ($\mu U \times ml^{-1} \times min^{-1}$ per 25 islets) released each min from islets during the 40 through 60 min of perifusion.

In experiments using [^{32}P]orthophosphate-labelled islets aliquots (0.2 ml) of perifusate were diluted with 10 ml 3a70B (Research Products International Corp., Elk Grove Village, Illinois) and counted in a Beckman LS-200B liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Release of [^{32}P]phosphate from islets was expressed as the percent of mean [^{32}P]phosphate from the 55th through the 60th min of perifusion.

Differences in total insulin and total [^{32}P]phosphate release during the "experimental period" (61–75 min) of perifusion were compared by analysis of variance [19].

Results

Figure 1 shows the effect of 20 mmol/l 2-DG in islet response to 8.3 mmol/l glucose. Insulin release in response to glucose was prompt but inhibited ($P<0.0005$) during the period of stimulation. Similarly 2-DG inhibited ($P<0.005$) insulin secretion stimulated by 16.7 mmol/l glucose (Fig. 2).

Islets that had been prelabelled with [^{32}P]orthophosphate for 90 minutes were perifused. When islets were exposed to 20 mmol/l 2-DG from the beginning of perifusion and then stimulated with 8.3 mmol/l glucose, inhibition ($P<0.005$) of [^{32}P]phosphate efflux was noted (Fig. 3). Similarly, the response to 16.7 mmol/l glucose was also inhibited ($P<0.01$) (Fig. 4). When islets were exposed to 20 mmol/l 2-DG following 60 minutes exposure to 2.8 mmol/l glucose, no release of [^{32}P]occurred (Fig. 5).

Discussion

The present studies showing the inhibitory effect of 2-DG on insulin release confirm previous findings [4, 7–11]. The higher the molar ratio of 2-DG to glucose in the medium, the greater is the inhibition [20]. To assist in determining how 2-DG may act to inhibit insulin release, we studied the effects of 2-DG on the glucose-induced heightened [^{32}P]phosphate efflux ("phosphate flush"). The "phosphate flush" is one of the earliest indices of stimulus-secretion coupling in islets and may be due to a membrane permeability change [12]. It is characterized by its stereo-specificity for α-D-glucose [21] and inhibition by mannoheptulose [22]. We have shown that 20 mmol/l 2-DG inhibits the "phosphate flush" induced by 8.3 or 16.7 mmol/l glucose, whereas 2-deoxyglucose alone does not induce the "phosphate flush". These findings in addition to those with mannoheptulose suggest that glucose metabolism may play a role in the induction of the "phosphate flush" as it does with calcium uptake. Islet uptake of calcium during the stimulus-secretion coupling is correlated with lactate output [23] and inhibited both by mannoheptulose and 2-DG [7]. Although these studies show the inhibitory effects of 2-DG on insulin and [^{32}P]phos-

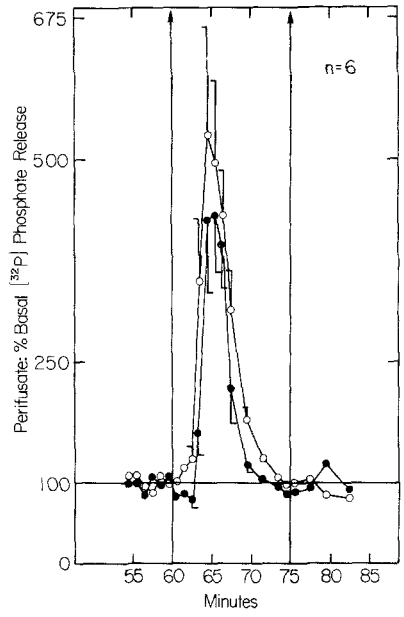

Fig. 4. Effect of 20 mmol/l 2-DG on the [^{32}P]phosphate efflux induced by 16.7 mmol/l glucose. Experimental design and presentation of results are as in Fig. 3, except that 16.7 mmol/l glucose was used instead of 8.3 mmol/l. [^{32}P]phosphate release from the first chamber corresponding to 100% basal was 287 ± 18 cpm × ml^{-1} × min^{-1} per 25 islets and from the second chamber 192 ± 19 cpm × ml^{-1} × min^{-1} per 25 islets. Each point represents the mean of 6 separate experiments. Vertical bars represent SEM

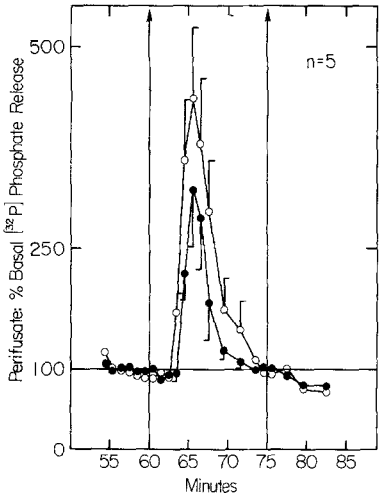

Fig. 3. Effect of 20 mmol/l 2-DG on the [^{32}P]phosphate efflux induced by 8.3 mmol/l glucose. Islets in the first chamber (○—○) were perifused with KRB-2.8 mmol/l glucose for the first 60 min, then with 8.3 mmol/l glucose from 61–75 min, and finally with 2.8 mmol/l glucose after 75 min. In the second chamber (●—●) islets were perifused with 20 mmol/l 2-DG and 2.8 mmol/l glucose for the first 60 min, then with 20 mmol/l 2-DG and 8.3 mmol/l glucose from 61–75 min, and finally with 2.8 mmol/l glucose. [^{32}P]phosphate release from the first chamber corresponding to 100% basal was 440 ± 36 cpm × ml^{-1} × min^{-1} per 25 islets and the second chamber was 211 ± 16 cpm × ml^{-1} × min^{-1} per 25 islets. Each point represents the mean of 5 separate experiments. Vertical bars indicate SEM

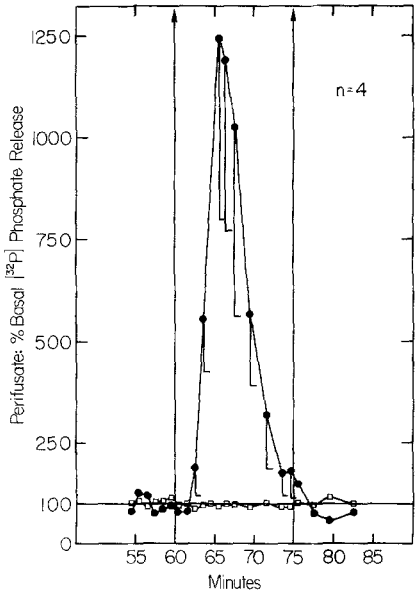

Fig. 5. Effect of 20 mmol/l 2-DG on [^{32}P]phosphate release. Both chambers were perifused with KRB-2.8 mmol/l glucose for the first 60 min. From 61–75 min the islets in the first chamber (●—●) were perifused with 16.7 mmol/l glucose while the islets in the second chamber (□—□) were perifused with 20 mmol/l 2-DG. After 75 min both chambers were perifused with 2.8 mmol/l glucose. [^{32}P]phosphate release from the first chamber corresponding to 100% basal was 312 ± 28 cpm × ml^{-1} × min^{-1} per 25 islets and from the second chamber 300 ± 21 cpm × ml^{-1} × min^{-1} per 25 islets. Each point represents the mean of 4 separate experiments. Vertical bars indicate SEM

phate release, they do not necessarily suggest a single site of inhibition common for both phenomena. The means by which glucose induces insulin release may involve both a direct glucoreceptor mechanism and glucose metabolism [24].

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