A Different Action of Hypothermia on Insulin Release from the Isolated, Perfused Rat Pancreas, Depending on the Stimulating Agent

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SUMMARY

Two series of experiments were performed in parallel on the isolated perfused rat pancreas. The experimental conditions differed only as pertaining to temperature. In one series the organ and the perfusion liquid were maintained at 37.5°C and in the other at 28°C. The pancreases were perfused from the start of the experiments with a perfusion medium containing 8.3 mmol/l glucose. The effects of various stimulatory agents were studied (glucose 16.6 mmol/l, tolbutamide 0.4 mmol/l, acetylcholine 0.5 μmol/l, glucagon, 2.8 nmol/l, and L-isoprenaline 0.05 μmol/l). At 37.5°C the insulin secretion induced by high glucose or tolbutamide, acetylcholine, and glucagon was biphasic and not statistically different. In all cases the hypothermia (28°C) decreased insulin secretion. However, glucose-induced and tolbutamide-induced insulin secretion was more decreased than the secretion induced by acetylcholine and glucagon. The study of the secretion ratios obtained at 28°C relative to 37.5°C showed that the ratios for the glucose and tolbutamide groups were significantly lower than those obtained for acetylcholine and glucagon groups for both the first and the second phase. The ratios were not significantly different between glucose and tolbutamide on the one hand and acetylcholine and glucagon on the other hand. In all groups the ratios 28°/37.5° for the second phase were lower than those obtained during the first phase. L-isoprenaline induced only a weak increase in insulin secretion and this was not long lasting; this increase was not statistically different at both temperatures. DIABETES 29:895–898, November 1980.

We have previously shown that the lowering of the temperature decreased insulin secretion from the isolated perfused rat pancreas but did not modify glucagon secretion.[1]

Our aim in the present work was to investigate whether hypothermia altered the insulin release induced by various stimulating agents in the same way. The selected temperature was 28°C in order to remain within the temperature range compatible with life in homeothermic animals.

MATERIALS AND METHODS

The pancreas was taken from Wistar rats, weighing about 350 g and fed ad libitum. The animals were anesthetized with sodium pentobarbital, and the pancreas was isolated and perfused as described previously.[2] The perfusion medium was a Krebs-Ringer bicarbonate buffer containing purified bovine albumin (2g/l) and glucose (8.3 mmol/l); it was bubbled with a mixture of O_2 (93%) and CO_2 (7%); the pH range was 7.27–7.34.

Two experiments were performed in parallel on the same day, and the experimental conditions differed only with respect to temperature. The pancreas and the perfusion fluid were maintained at 37.5°C or at 28°C. The flow rate ranged from 2.2 to 2.6 ml/min. The arterial perfusion pressure was on average 35 cm H_2O (range, 31–40) at 37.5°. In the experiments at 28°, it was on average 2.5 cm higher (range, 33–43). In all cases, a 30-min equilibration period was allowed between the beginning of perfusion and the first sample taken for insulin assay. One more sample was taken 15 min later. The stimulating agents were then added. On the figures, time is counted from the beginning of organ perfusion.

Insulin was assayed in the effluent from the pancreas using the radioimmunologic method B of Hales and Randle[3] with pure rat insulin from Novo Laboratories as the standard.

Various substances were studied: D-glucose, tolbutamide, acetylcholine, glucagon, and L-isoprenaline. The kinetics of insulin output were studied for each agent and for each temperature.

Statistical analysis. In each experiment, three periods were studied: the control period before adding the stimulating agent, the stimulation first phase (4 min) and the stimulation second phase (the next 26 min).

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The insulin output during one minute at time 45 min was taken as the reference insulin output rate (RIOR) (ng/min). For the control period (30–45 min), the stimulation first phase, and the stimulation second phase, the total amount of insulin collected in each period was calculated. This amount, divided by the collection time, gave us the mean insulin output rate (MIOR). For the first and the second phase, the mean additional insulin output rate (MAIOR) was calculated as MIOR–RIOR.

The ratio 28°C/37.5°C expresses the fraction recorded at 28°C relative to 37.5°C under each condition. This ratio was calculated using the paired experiments carried out on the same day.

The statistical study of the data was performed using the analysis of variance. When the groups were significantly different, we used the method of multiple comparisons of Student–Newman–Keuls.[4] The two-tailed *t* test of Student was used for the values obtained under the effect of isoprenaline.

RESULTS

Effects of glucose, tolbutamide, acetylcholine, and glucagon. The stimulating action was about the same for glucose 16.6 mmol/l, tolbutamide 0.4 mmol/l, acetylcholine 0.5 μmol/l, and glucagon 2.8 nmol/l when the temperature was 37.5°C (Figure 1). The response was biphasic, with an immediate sharp phase followed by a second durable phase. The response to these different agents was quite different at 28°C (Figure 1): in both secretory phases the insulin release induced by acetylcholine and glucagon was much higher than that induced by glucose and tolbutamide.

In Table 1 are the values of MIOR during the control period and the RIOR, as well as the MAIOR during the first and second phase of stimulation at 37.5°C and 28°C. At 37.5°C, no statistically significant difference was seen among the four series of experiments. In contrast, the variance analysis showed that hypothermia modified differently the mean additional insulin output rates, depending on the stimulating agent ($P < 0.001$). Those were much lower with glucose and tolbutamide than with acetylcholine and glucagon.

The study of the secretion ratios obtained at 28°C relative to 37.5°C (Table 1) showed that the four groups were not statistically different for the control period and at time 45 min. With regard to the MAIOR, the ratios for the glucose and tolbutamide groups were significantly lower than those obtained for the acetylcholine and glucagon groups for both the first and the second phase ($P < 0.025$). The ratios were not significantly different between glucose and tolbutamide on the one hand and acetylcholine and glucagon on the other hand.

In all groups the ratios 28°/37.5° for the second phase were lower than those obtained during the first phase.

FIGURE 1. Effect of various stimulatory agents on insulin secretion induced by glucose 8.3 mmol/l. In three series of experiments, tolbutamide, acetylcholine, or glucagon were infused for 30 min (from 45 min to 75 min). In one series, 8.3 mmol/l glucose was replaced by 16.6 mmol/l glucose from time 45 min. Left panel: effect at 37.5°C. Right panel: effect at 28°C. Results are expressed as mean ± SEM, and N = number of experiments.

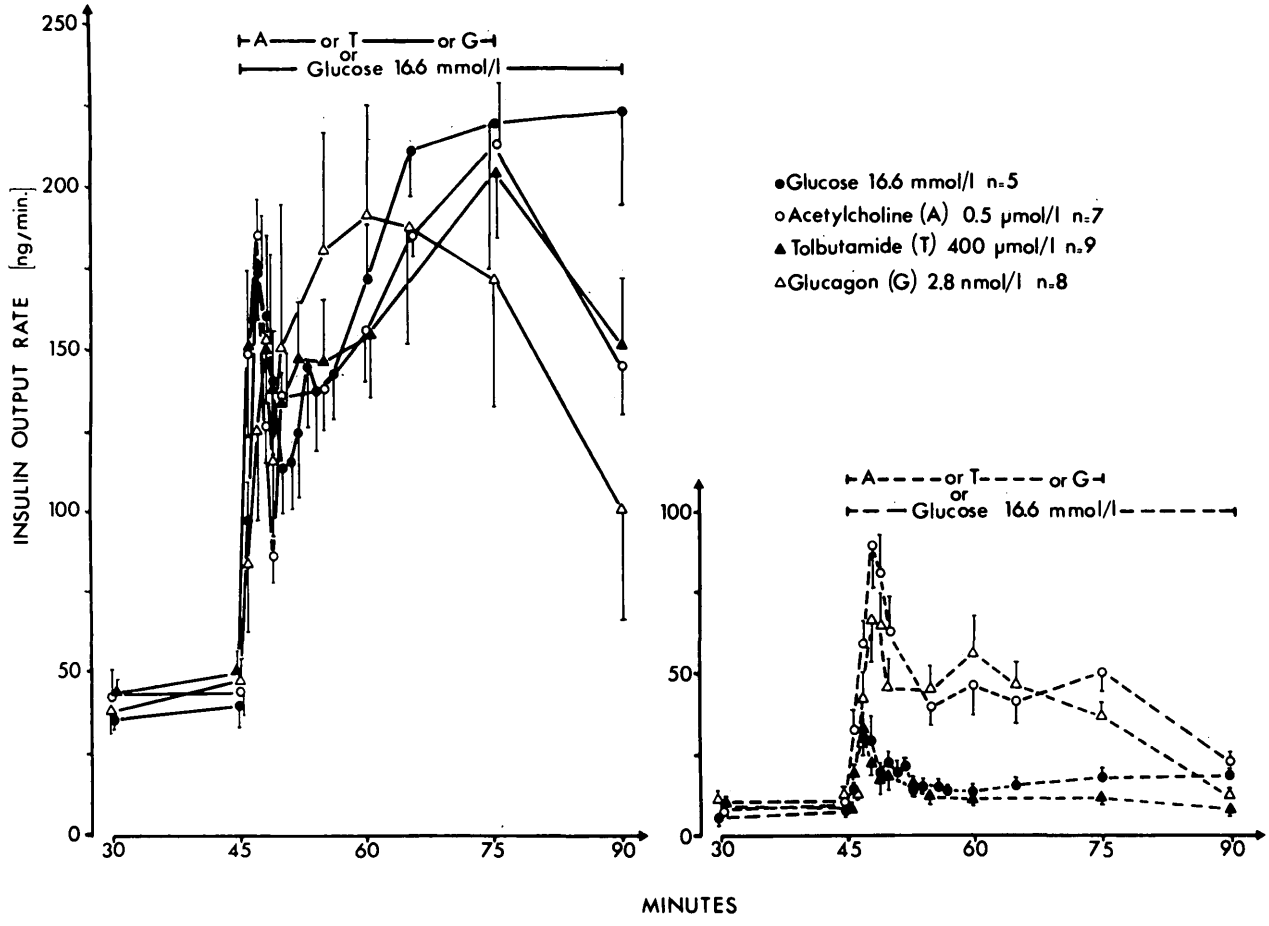


TABLE 1
Values of insulin secretion induced by various stimulatory agents at 37.5°C and 28°C. Insulin output rate before adding these agents and the additional insulin output rate elicited by them are given in nanograms per minute. Ratio 28°C/37.5°C expresses the fraction recorded at 28°C relative to 37.5°C under each condition (see text). N = number of experiments.

Stimulating agents		Control period	Reference insulin output rate	First phase mean additional insulin output rate	Second phase mean additional insulin output rate
37.5°C					
Glucose	(16.6 mmol/l) N = 5	37.8 ± 3.8	40.1 ± 6.1	103.9 ± 13.0	137.6 ± 16.5
Tolbutamide	(0.4 mmol/l) N = 9	46.9 ± 6.2	51.8 ± 6.4	102.6 ± 14.7	105.3 ± 17.9
Acetylcholine	(0.5 μmol/l) N = 7	43.4 ± 6.3	44.0 ± 7.5	93.1 ± 7.8	124.3 ± 10.7
Glucagon	(2.8 nmol/l) N = 8	43.3 ± 8.2	48.0 ± 9.5	70.8 ± 16.1	126.8 ± 26.8
Isoprenaline	(0.05 μmol/l) N = 7	38.1 ± 3.6	41.3 ± 4.2	10.6 ± 2.1	7.2 ± 4.5
28°C					
Glucose	(16.6 mmol/l) N = 5	6.5 ± 0.8	7.6 ± 1.1	15.6 ± 3.3	8.8 ± 2.1
Tolbutamide	(0.4 mmol/l) N = 9	8.4 ± 1.1	8.1 ± 1.1	14.3 ± 2.7	4.9 ± 1.8
Acetylcholine	(0.5 μmol/l) N = 7	8.5 ± 1.4	8.3 ± 1.9	55.9 ± 6.5	37.1 ± 5.3
Glucagon	(2.8 nmol/l) N = 8	10.2 ± 2.0	10.2 ± 2.0	36.3 ± 6.1	34.9 ± 5.5
Isoprenaline	(0.05 μmol/l) N = 7	8.4 ± 1.8	8.9 ± 2.0	10.8 ± 2.0	3.1 ± 1.4
Ratio 28°C/37.5°C					
Glucose		0.17 ± 0.02	0.19 ± 0.02	0.17 ± 0.04	0.06 ± 0.01
Tolbutamide		0.22 ± 0.06	0.22 ± 0.02	0.14 ± 0.02	0.06 ± 0.03
Acetylcholine		0.20 ± 0.03	0.21 ± 0.03	0.62 ± 0.08	0.30 ± 0.04
Glucagon		0.28 ± 0.06	0.27 ± 0.06	0.74 ± 0.20	0.40 ± 0.11
Isoprenaline		0.23 ± 0.06	0.23 ± 0.06	—	—

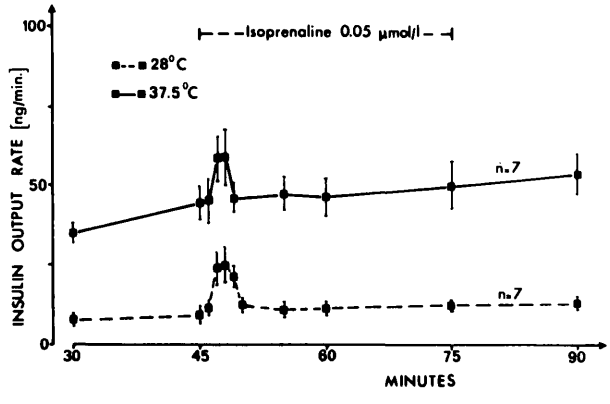
Effects of isoprenaline. For the control period and at time 45 min the insulin output rates and the ratios did not significantly differ from the other groups (Table 1). Isoprenaline induced an additional insulin release only for the first 4 min at 37.5°C and 28°C, and it was not statistically different at either temperature (Table 1 and Figure 2).

DISCUSSION

Lowering of the temperature strongly decreased the insulin output. However the effect was not the same with all the stimulating agents. In our experiments, the concentrations of glucose, tolbutamide, acetylcholine, and glucagon were chosen to obtain a similar increase when the temperature was 37.5°C. Glucose-induced and tolbutamide-induced insulin secretion was decreased by hypothermia more than the secretion induced by acetylcholine and glucagon.

The insulin stimulatory action of sugars seems to be related to their metabolic activity.[6-8] As the lowering of the temperature usually decreases the metabolism of organs, a possible mechanism for hypothermia-induced suppression of insulin release may, therefore, be through a decrease in B-cell glucose metabolism. It is well established that acetylcholine acts on membrane receptors; it is possible that the membrane phenomena would be less modified by the temperature than the metabolic events. Glucagon acts on the cellular membrane and increases the adenylcyclase activity. According to Pradhan and Criss,[9] activity of normal liver adenylate cyclase is clearly decreased only below 25°C. The biochemical events that lead to the insulinotropic action of sulfonylureas are not yet known. In our experiments the hypothermia modified the insulin release induced by these agents in the same way as the glucose-induced insulin release.

FIGURE 2. Effect of isoprenaline on insulin secretion at 37.5°C and 28°C induced by 8.3 mmol/l glucose. Results are expressed as mean ±SEM, and N = number of experiments.



As previously shown,[5] L-isoprenaline elicited only a weak and short stimulation which was not modified by hypothermia. It is known that this catecholamine stimulates the β-adrenoreceptors and activates adenylate cyclase.

In all cases the late phase is more decreased by hypothermia than is the early phase. This might be explained by the fact that energy production and glucose metabolism are needed for long-lasting stimulation and there is a reduction in all metabolic and energy-requiring processes during hypothermia. Furthermore, the rate of proinsulin synthesis and conversion to insulin was shown to depend on temperature,[10] and newly synthesized insulin plays a part in the late insulin response.[11]

With regard to technical conditions, our results allow us to call attention to the fact that close control of temperature in studies on perfused pancreas or perifused islets is rather critical.

In conclusion, our results show differences in inhibition by hypothermia of the insulin response to various stimuli

and that in all cases the second phase is more decreased than the first phase.

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