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Positive Inotropic Action of Insulin on Piglet Heart[1]

ROBERT P. RIEKER,[2] JOHN C. LEE, AND S. EVANS DOWNING[3]

Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510

Piglets of 1 day to 12 weeks of age were prepared for evaluation of myocardial contractility by measurement of left ventricular dP/dt max under constant hemodynamic conditions. Practolol (1 mg/kg) was given to each animal to provide beta adrenergic blockade. In 12 piglets given crystallin insulin (40 units), arterial glucose fell from 102 to 22 mg% after 75 min. This was accompanied by an increase of dP/dt max to 138% of initial values within 30 min. The positive inotropic effect persisted for the duration of the study. Eight control animals and two piglets given glucagon (50 μg/kg) showed no significant change. After the 75-min study period, each animal was made hypoxic by ventilation with N_2. The time of onset and severity of hypoxic left heart failure in piglets given insulin did not differ from control animals. It is concluded that insulin augments myocardial contractility in the piglet and that this property is independent of adrenergic mechanims or glucagon contamination. Insulin administration apparently does not diminish cardiac depression engendered by acute hypoxia.

Insulin has long been recognized to have a number of effects on the cardiovascular system. Bayliss *et al.* demonstrated that the heart–lung preparation was maintained in good condition for longer periods with the addition of glucose and insulin to the perfusate (1). They also showed that insulin-induced hypoglycemia increased the frequency of contractions of the denervated heart. Ernstene and Altschule noted an increase in pulse and systolic pressures and cardiac output in human subjects made hypoglycemic by insulin (2). These changes were thought to be consequent to sympathetic stimulation engendered by the hypoglycemia, a view consistent with the concommitant increase in plasma epinephrine reported by Wallore *et al.* (3) in similar studies. An additional mechanims has been reported by Perda *et al.* These workers described a pressor response when small doses of insulin were injected into the carotid arteries of dogs that did not become hypoglycemic (4). They concluded that this probably represented a direct effect of insulin upon the CNS.

The foregoing studies suggest that insulin elicits positive inotropic and chronotropic effects on the heart that may be attributed to sympathetic stimulation resulting from hypoglycemia and a direct hormonal action on the CNS. However, the observations of Visscher and Muller, who showed that the addition of insulin to the circulating blood in a heart–lung preparation "results in a decrease in volume of the ventricles of the heart," suggest that extrinsic adrenergic pathways may not be fully responsible for the observed hemodynamic effects (5). A direct action of insulin upon the myocardium is therefore a likely additional mechanism.

The influence of insulin of the performance of developing postnatal myocardium has not been previously examined. This study was designed to explore the direct effects of the hormone using the piglet model. Beta blockade was used to separate

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[2]Present address: R. P. Rieker, M.D., Bowman Gray Medical School, Department of Pediatrics, Winston-Salem, N.C.

[3]Send requests for reprints to S. Evans Downing, M.D., Department of Pathology, Yale University School of Medicine, 310 Cedar St., New Haven, Conn. 06510.

adrenergic from nonadrenergic mechanisms. In addition, the tolerance of insulin-treated animals to hypoxia was compared with a control group.

METHODS

Twenty piglets ranging in age from 1 day to 12 weeks and weighing 1–16 kg were studied. All animals were fasted for 16–24 hr. The animals were anesthetized by intraperitoneal sodium pentobarbital (25 mg/kg). A tracheostomy was performed, and ventilation was maintained by a Harvard constant-volume positive pressure ventilator. The external jugular vein was cannulated.

The essential features of the preparation are illustrated in Fig. 1. Following heparinization (500 units/kg), the descending thoracic aorta was cannulated. Aortic flow was measured with a Statham 6.0-mm o.d. extracorporeal flow transducer and a Medicon K-2000 electromagnetic flow meter. The flow transducer was calibrated *in vitro* with normal saline. Aortic flow was then passed through a Sarns heat exchanger and returned to the descending aorta. Cephalic blood flow was abolished by ligating the brachiocephalic and left subclavian arteries. Systemic flow was controlled with a roller pump, and arterial pressure was controlled with an adjustable constant pressure reservoir in the extracorporeal circuit. The system was primed with fresh heparinized (5 mg/100 ml) donor swine blood. Blood temperature was maintained at 38 ± 1°C with the heat exchanger system and continuously monitored with a Yellow Springs probe and telethermometer. Arterial pH, PO_2 and PCO_2 were

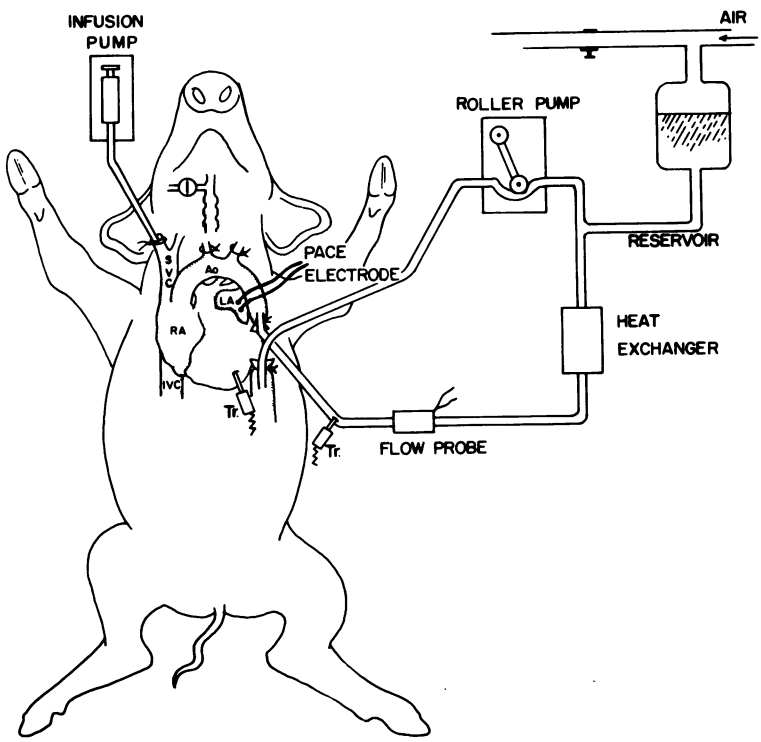

FIG. 1. Preparation for measurement of left ventricular performance in newborn piglet. Brachiocephalic and subclavian arteries ligated. Aortic flow measured with cannulating type electromagnetic flow probe. Systemic blood flow held constant with roller pump. TR. = Sanborn transducer. See text for further description of preparation.

monitored with a calibrated Instrumentation Laboratories blood gas analyzer and pH system. Glucose concentration was determined by the Glucostat quantitative enzymatic determination of glucose (Worthington Biochemical Co.). Arterial pH was maintained in the normal range (7.30–7.45) by infusion of $NaHCO_3$ when necessary. Hematocrit remained at a level of 25–35%. All solutions infused or injected were free of glucose.

Left ventricular pressure was measured by means of a 15-gauge needle passed through the apex into the left ventricular cavity. Aortic and ventricular pressure measurements were made with Sanborn transducers (267 series). The midlevel of the heart was used as zero reference. The maximum rate of rise of left ventricular pressure (dP/dt max) was obtained using a R-C differentiating circuit with a time constant of 0.269 msec. Heart rate was controlled by electrically pacing the left atrium with a Grass-SD-5 stimulator (Fig. 1). The pressures, aortic flow, heart rate, and left ventricular dP/dt measurements were recorded simultaneously on a multichannel oscillograph (Sanborn model 358) at chart speeds of 0.25 or 100 mm/sec.

Practolol (1 mg/kg) was administered to each animal. This blocked the inotropic response to a test bolus of isoproterenol (0.1 μg) given to all animals during and at the end of each experiment. Following baseline sampling and recording, Group I (12 animals) received 40 units (0.5 ml) of crystallin insulin (Eli Lilly Co.), intravenously. Group II (8 animals) received 0.5 ml of normal saline. Both groups were observed for 75 min. Samples were obtained every 15 min. Seventy-five minutes after the control point both groups were subjected to hypoxia. This was achieved by reducing the O_2 concentration in the ventilator by using a Simet gas mixer. Sampling and recordings were made every 5 min and at the end point. The end point of hypoxia was taken as onset of depression of myocardial function manifested by rapidly decreasing dP/dt max, rising LVEDP, and decreasing aortic flow and pressure. Two additional piglets were studied to assess responsiveness to glucagon (Lilly), 50 μg/kg.

RESULTS

Effect of Insulin on Arterial Glucose Concentration

The 12 piglets given insulin demonstrated a progressive decrease in arterial glucose concentration from an initial value of 102 ($\pm$ 12 SE) mg% to 66 ($\pm$ 10 SE) mg% at 30 min ($p < 0.05$) (Fig. 2A). Seventy-five minutes after insulin it was 22 ($\pm$ 4 SE) mg%. These animals were subdivided into two groups that included six less than 1 week and six more than 1 week of age. The initial mean glucose concentration for the younger groups was 109 ($\pm$ 18 SE) mg%. As shown in Fig. 2B, 30 min following insulin it decreased to 72 ($\pm$ 17 SE) mg% and reached a level of 22 ($\pm$ 7 SE) mg% at 75 min. Those animals older than 1 week had a slightly lower initial glucose concentration of 96 ($\pm$ 14 SE) mg%. This fell to 60 ($\pm$ 10 SE) mg% 30 min after insulin, and by 75 min was 23 ($\pm$ 6 SE) mg%. Thus, the hypoglycemic response to insulin did not differ significantly within this age range.

Initial glucose values in the eight control animals (Fig. 2A) were slightly higher 111 ($\pm$ 16 SE) mg% than the insulin-treated group (102 mg%). After 75 min the mean value fell to 79 ($\pm$ 15 SE) mg%, but this was not statistically significant (p = 0.25). This value did not differ from the mean obtained prior to insulin in the treated animals.

Inotropic Response to Insulin

The animals given insulin demonstrated a mean increase in dP/dt max to 138 ($\pm$ 7 SE) % of initial values at 30 ($p < 0.01$). At 75 min dP/dt max in the insulin group

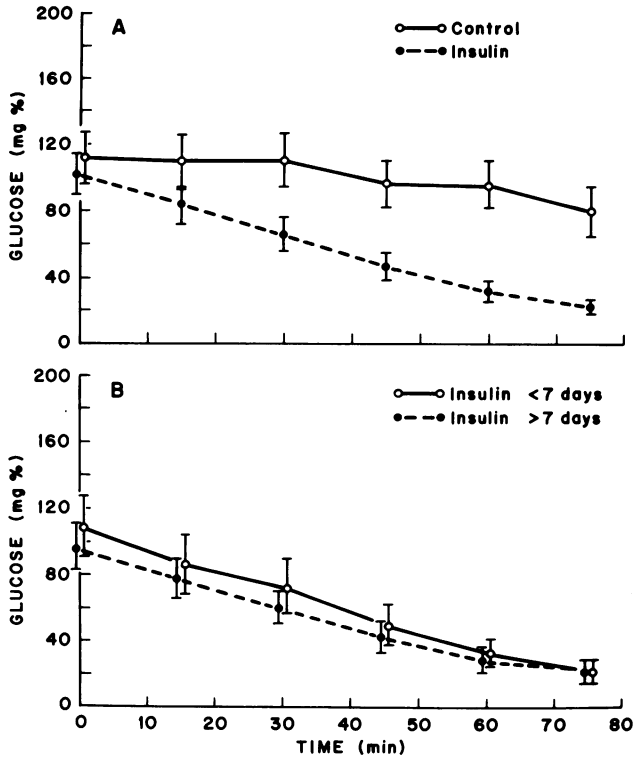

FIG. 2. (A) Arterial glucose concentrations obtained at 15-min intervals with standard errors obtained from eight control piglets (open circles) are compared with 12 animals given 40 units of insulin. The small reduction in mean glucose concentrations in the control animals was not statistically significant. Insulin-treated animals differed significantly from controls after 30 min. (B) Comparison of arterial glucose concentrations following insulin administration in piglets less than 1 week of age with older animals. No evidence for an age-related difference was detected.

was 137 ± 8 SE) % of original levels (Fig. 3). The average values for LVEDP tended to be less following insulin, though the differences were not significant. Thus, as shown in Table 1, the increase in contractility reached a maximum by 30 min and remained nearly constant over the remaining 45 min. The increase of dP/dt max in animals younger than 1 week did not differ from those older than 1 week ($p > 0.25$). The control animals, on the other hand, demonstrated no significant change in dP/dt max over the same time group, while the mean values for LVEDP rose progressively (Table 1). After 75 min dP/dt max in this group was 106 (± 6 SE) % of initial values ($p > 0.50$) (Fig. 3). Piglets given glucagon failed to manifest a significant change in cardiac performance.

Response to Hypoxia

Following the intial 75-min study period, each aminal was made hypoxic by replacing the O_2 in the respirator with N_2. At the point of initiation of N_2, the only parameter measured other than dP/dt max was significantly different between the groups was the arterial glucose level (Table 1).

The hemodynamic responses to hypoxia are summarized in Table 1 and showed no detectable differences between the two groups. The time from onset of hypoxia to the end point was 8.8 (± 1.8 SE) min for the insulin treated against 9.3 (± 1.7 SE)

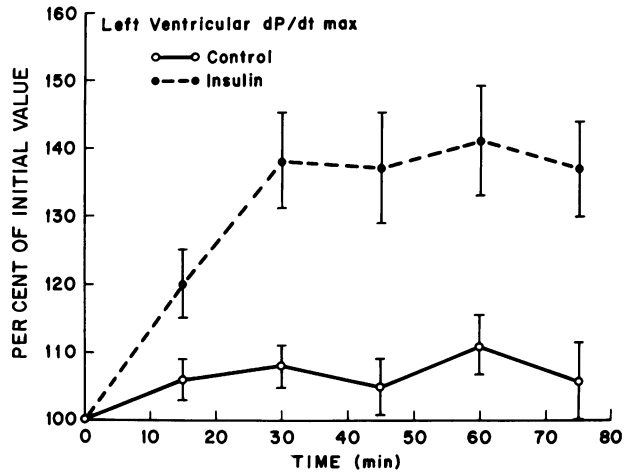

FIG. 3. Changes in left ventricular dP/dt max expressed in terms of percentage of values obtained immediately prior to insulin administration. Vertical bars indicate standard error of mean. Measurements obtained under conditions of constant heart rate (paced), aortic pressure, and systemic blood flow. Following insulin (closed circles) dP/dt max increased and stabilized after 30 min at approximately 140% of initial values. Control animals showed no significant difference from initial values.

min for control ($p > 0.50$). The LVEDP for insulin groups was 16.7 (± 2.7 SE) cm H_2O, as compared with 18.8 (± 3.7 SE) cm H_2O for the controls ($p > 0.25$). Therefore, under the conditions of these experiments, insulin-induced hypoglycemia did not appear to alter the myocardial response to hypoxia.

DISCUSSION

The objective of this study was to assess changes in the performance of newborn hearts during insulin-induced hypoglycemia that may be attributed to non-

TABLE 1[a]

	Time (min)						
	0	15	30	45	60	75	E.P.
				Group I (control)			
Glucose	111 ± 16	109 ± 16	110 ± 16	95 ± 14	95 ± 14	79 ± 15	75 ± 16
PaO_2	158 ± 26	214 ± 32	192 ± 39	154 ± 31	156 ± 33	130 ± 32	25 ± 2
dP/dt max	1612 ± 147	1690 ± 146	1722 ± 142	1690 ± 146	1762 ± 158	1750 ± 150	1089 ± 120
LVEDP	4.0 ± 0.4	4.5 ± 0.7	4.9 ± 0.8	4.9 ± 0.8	5.1 ± 0.8	5.9 ± 1.2	18.8 ± 3.7
Time to E.P. (min)							9.3 ± 1.7
				Group II (insulin treated)			
Glucose	102 ± 12	82 ± 10	66 ± 10	46 ± 8	31 ± 6	22 ± 4	19 ± 4
PaO_2	159 ± 15	157 ± 26	213 ± 54	178 ± 39	192 ± 30	144 ± 29	30 ± 4
dP/dt max	1491 ± 159	1741 ± 131	1968 ± 131	1945 ± 148	2010 ± 130	1940 ± 130	1234 ± 140
LVEDP	4.7 ± 1.1	4.3 ± 1.0	4.1 ± 1.0	3.6 ± 1.1	3.6 ± 1.2	4.0 ± 1.0	16.7 ± 2.7
Time to E.P. (min)							8.8 ± 1.8

[a]Values are means ± SEM. 0 time indicates values obtained immediately prior to injection of saline or insulin. 15, 30, 45, 60, and 75 min are time intervals from 0 time. E.P. = end point of induced hypoxia as defined in text. dP/dt max in mm Hg/sec. Glucose, mg%. PaO_2 = mm Hg. LVEDP = cm H_2O.

adrenergic mechanisms. Beta blockade was achieved by the use of practolol. Persistance of the block was shown by repeating the test bolus of isoproterenol during and at the end of each experiment. Hence, the observed positive inotropic effects of insulin were likely secondary to other factors. Presumably these would include a direct effect upon the myocardium, which may involve membrane transport, metabolism, or other subcellular mechanisms such as cation, especially calcium, fluxes.

The preparation used was commercially available crystallin insulin, 80 units/ml. It may contain up to 0.1% impurity, which if biologically active might contribute to the inotropic effect. Indeed, Glasgow found a hyperglycemic response when he injected large doses (15 units-kg) into rabibts, presumably secondary to glucagon contamination of the insulin preparation (6). Glucagon has a positive inotropic effect on adult cardiac tissue, which is rapid in onset (less than 5 min) and short in duration (less than 30 min) (7). Despite the relatively large dose of insulin used (40 units), we did not detect an initial hyperglycemic response. Probably this is because the preparation used (Lilly) would be expected to contain only about 7 μg of glucagon in each dose or less than 4 μg/kg in most animals. Two additional piglets given as much as 50 μg/kg of glucagon, iv, failed to manifest a positive inotropic response, a finding that is similar to that with the newborn lamb (8). Moreover, when compared with the known effects of glucagon in the adult mammal, the increase in dP/dt max following insulin was not as rapid in onset, did not become maximal until 30 min, and continued for the full duration of 75 min. It is therefore unlikely that the observed inotropic effect was the result of glucagon contamination of the insulin preparation.

Studies with adult myocardium have generally failed to show a positive inotropic response from insulin in either the intact (9) or isolated (10) muscle system unless depressed by another intervention. For example, Lucchesi *et al.* (10) reported a positive response to insulin in isolated canine papillary muscle preparations, but only when the hormone was given after prolonged incubation (17 hr) associated with a marked reduction in maximal isometric tension development. The control animals in our study showed no evidence for significant deterioration during the full duration of the experiment (Fig. 3). The observation that insulin produces a pronounced improvement in cardiac performance of hearts depressed with halothane is also consistent with the above interpretation (11). It is pertinent to note that barbiturates do not manifest this effect (10).

Numerous studies support the concept that the glycogen content of neonatal and adult myocardium influences its resistance to hypoxia (12–15). Theoretically, if one could increase the glycogen content and therefore increase substrate for anaerobic metabolism, tolerance to hypoxia would increase. Chain and associates concluded from studies with perfused rat hearts that stimulation of glucose metabolism by insulin cannot be explained by simple acceleration of glucose transport but is the consequence of specific stimulation of glycogen synthesis (16). This suggests that more pretreatment with insulin might protect the myocardium from hypoxia. However, in the present study no differences were detected between the control group and those given insulin when exposed to hypoxia (Table 1). The times to "failure" level of LVEDP and PaO_2 at the end point were almost identical. Moreover, these findings are consistent with recent observations in the isolated rat heart preparation reported by Gmeiner *et al.* (17).

While the foregoing hypothesis was not supported by these experiments, it should be noted that myocardial glycogen concentrations were not determined and may not

have differed in the two groups. Secondly, it is possible that practolol used in these studies interfered with phosphorylase activation, as has been shown with pronethalol (18). This explanation would seem unlikely, however, in view of the similar findings by Gmeiner, *et al.* (17), where these agents were not employed. It is clear from this study that administration of insulin to the newborn piglet with normal oxygenation is accompanied by a large and sustained augmentation of contractility that is unrelated to adrenergic stimulation. The mechanisms responsible for this effect remain to be elucidated. But the improvement in cardiac performance that is demonstrable during normoxemia apparently is not sustained if the myocardium is made acutely hypoxic.

SUMMARY

This study was designed to investigate changes in cardiac performance during hypoglycemia produced by the administration of insulin in the newborn piglet. With heart rate, aortic pressure, and aortic flow held constant, the treated group demonstrated a pronounced positive inotropic response manifested by an increase of dP/dt max to 138% of control values. Central nervous system function and beta adrenergic activity were excluded from the preparation by ligation of the brachiocephalic vessels and administration of practolol. For reasons discussed, it is unlikely that the findings can be ascribed to glucagon contamination. Therefore, the increase in contractility presumably resulted from a direct effect of insulin upon the myocardium. Clinical and laboratory data suggest that the resistance of the neonate to hypoxia is modified by glycogen stores. Insulin is known to increase glycogen synthesis, and this effect might be expected to augment myocardial resistance to hypoxia. Under the conditions of these experiments, however, pretreatment with insulin had no demonstrable influence on the rate of deterioration of cardiac function during hypoxia. The mechanism of cardiac stimulation by insulin is unknown but may involve calcium fluxes.

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