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COMPARATIVE EFFECTS OF INSULIN AND PROINSULIN IN VITRO ON PATHWAYS OF GLUCOSE UTILIZATION AND LIPID SYNTHESIS IN THE LACTATING RAT MAMMARY GLAND

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The effects of insulin and proinsulin have been measured on the rates of glucose oxidation via the pentose phosphate pathway and incorporation into lipid in slices of lactating rat mammary gland. Half-maximal stimulation of glucose oxidation was observed with 1-3 x 10^{-8}M insulin while 1 x 10^{-7}M proinsulin was required to achieve half-maximal stimulation. A similar, approximately 10-fold, difference in potency was observed in relation to lipid synthesis. The present results appear to indicate that the maximum stimulation of either glucose oxidation via the pentose phosphate pathway or lipid synthesis by proinsulin did not reach the same level as that found for insulin.

There has been extensive work on the effects of insulin in the control of mammary gland metabolism, including a wide range of studies on the in vitro effects of insulin on the metabolism of glucose by lactating mammary gland slices, isolated acini and isolated cells [1-6]. It has been shown that addition of insulin in vitro to lactating rat mammary gland slices incubated with specifically labelled glucose results in a marked increase in the flux of glucose via the pentose phosphate pathway [PPP] and of glucose incorporation into lipid [1,2,5].

Recent studies have shown that proinsulin, the precursor of insulin, has an insulin-like action on the liver, adipose tissue and diaphragm [7-11], the potency of proinsulin being considerably less than that of insulin, the half-maximal effective concentrations of proinsulin have been reported as being between 10-100 times greater than that of insulin on parameters measured in hepatocyte preparations, including the regulation of glycogen synthesis, gluconeogenesis and ketogenesis [8-10]. The potency of human insulin and proinsulin was shown to be equally effective in the suppression of the glucagon-dependent stimulation of glycogenolysis [7]. Proinsulin was even less effective compared to insulin when evaluated by the ability to stimulate glucose incorporation into lipid and on antilipolytic effects in isolated

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adipocytes; on both parameters proinsulin possessed less than 1% of the potency of insulin; a similar 100-fold difference in receptor binding to adipocytes was also observed [10]. However, human proinsulin has been reported to have approximately 10% of the binding and biological potency of insulin in adipocytes, the latter being evaluated by the effect on 2-deoxyglucose transport [9].

In view of the difference in potencies of proinsulin relative to insulin in different tissues, it was of interest to study the potency of these two hormones on the glucose metabolism of the mammary gland for the following reasons: (i) there is a reported biphasic change in the level of plasma immunoreactive insulin during the lactation cycle, with a rise during pregnancy and a fall in the switch-over period from late pregnancy to the highly biosynthetic state of lactation [12,13]; (ii) it has been reported that there is an increased level of circulating proinsulin in diabetic pregnant women [14] and in some hormonal conditions [15]; (iii) inter-organ regulation by hormones is a critical factor in the direction of metabolites to biosynthetic purposes in lactation [16] and insulin and proinsulin could play a role in this complex regulation, particularly if there was a differential sensitivity among organs to these two hormones.

The present study investigates the relative sensitivity of rat mammary gland, at the mid-lactation stage, to insulin and proinsulin with respect to the oxidation of glucose via the PPP and the incorporation of glucose into lipid.

MATERIALS AND METHODS

Materials. Biosynthetic human proinsulin was a gift from Eli Lilly (Lilly Research Centre, Windlesham, UK) and porcine insulin was purchased from the Sigma Chemical Co. (Poole, UK). The concentrations of insulin and proinsulin were calculated on the basis of the values given by Cohen et al. [17].

Glucose labelled on either carbon-1 (C1) or carbon-6 (C6) was obtained from the Radiochemical Centre (Amersham, Bucks, UK).

Animals. Primiparous female rats of the Wistar strain were used on the 10th day of lactation; the litter size was restricted to 8-10 pups. Tissue slices were prepared from the abdominal mammary glands using a Stadie-Riggs tissue cutter as previously described [1,5].

Measurement of glucose flux rates. The rate of formation of $^{14}CO_2$ from glucose labelled either on carbon-1 or carbon-6 by lactating rat mammary gland slices, and the incorporation into ^{14}C-lipid, in the presence of increasing concentrations of insulin or proinsulin ($10^{-8}$to 10^{-6}M), was measured as previously described [1,5].

The activity of the pentose phosphate pathway (PPP) was evaluated from the difference between the incorporation of C1-C6 into $^{14}CO_2$; the problems inherent in the quantitative calculation of the PPP have been discussed by Katz et al. [18,19].

The results are given as means ± SEM; Fisher's P values are given for comparisons between control tissue slices without hormone against those with increasing concentration of insulin and proinsulin (Table 1).

RESULTS AND DISCUSSION

The effects of insulin and proinsulin *in vitro* on glucose utilization and lipid synthesis by mammary gland slices prepared from 10-day lactating rats are shown in Table 1.

Effect of insulin and proinsulin on glucose oxidation.

Insulin increased the conversion of glucose labelled on carbon-1 to $^{14}CO_2$, the maximum response of +78% was obtained at 1.5 x 10^{-7}M and raising the concentration to 1 x 10^{-6} had no further stimulatory effect. The half-maximal effect was obtained with concentrations of approximately 1 x 10^{-8}M. In contrast, proinsulin at 1 x 10^{-6}M caused only a +40% increase in carbon-1 oxidation and, at 1 x 10^{-8}, no significant increase was observed. The effect of proinsulin appeared to reach a plateau at a concentration of 5 x 10^{-7}M with half maximal effects at approximately 1 x 10^{-7}M. This would indicate a 10-fold difference in the response of tissue slices from lactating rat to insulin and proinsulin, i.e. in accord with the relative sensitivities reported for liver on ketogenesis, gluconeogenesis and glycogen synthesis by Agius et al. [8] and for glucose transport in adipocytes by Podlecki et al. [9]. However, the present data differ from those reported for hepatocytes and adipocytes in that, even with very high non-physiological concentrations of proinsulin , the maximum stimulation of conversion of C1 of glucose to $^{14}CO_2$ is significantly lower than with insulin. The addition of the protease inhibitor, trasylol, to the incubation medium did not alter, in any significant degree, the effect of insulin and proinsulin. It is possible that with even higher concentrations of proinsulin a plateau value similar to that found with insulin *in vitro* might be reached, but such excessively high concentrations were not tested in the present experiments.

The quantitative evaluation of the PPP is complex in that recycling produces unlabelled glucose 6-phosphate to dilute the radioactive pool of glucose 6-phosphate and an enrichment of the triose phosphate pool by carbons 4,5 and 6 of glucose which are not oxidized in the PPP. The C1 - C6 value has been broadly accepted as indicating the direction of change of the PPP. In the present experiments, it is used as a comparative, rather than a quantitative, parameter for studying the relative effects of insulin and proinsulin.

As shown in Table 1, there is significant decrease in the conversion of C6 of glucose to $^{14}CO_2$ by mammary gland slices incubated in the presence of insulin or proinsulin. The magnitude of the effect is approximately the same for the two hormones, i.e. a 50% decrease. The effect of insulin in depressing

Table 1. Effect of insulin and proinsulin *in vitro* on glucose utilization and lipid synthesis by mammary gland slices prepared from 10-day lactating rats

	$^{14}CO_2$ yields from ^{14}C-glucose (μmol/g/hr)			^{14}C-lipid formation from ^{14}C-glucose (μmol/g/hr)	
	[1-^{14}C] glucose	[6-^{14}C] glucose	PPP (C_1-C_6)	[1-^{14}C]-glucose	[6-^{14}C]-glucose
EFFECT OF INSULIN					
Concⁿ insulin (M)					
0	26.7 ± 0.6 (32) 100 %	1.77 ± 0.08 (19) 100 %	24.9 ± 0.7 100 %	21.9 ± 0.6 (32) 100 %	33.1 ± 1.2 (18) 100 %
1 x 10^{-8}	36.6 ± 1.7 (13) 137 % ***	1.59 ± 0.19 (7) 90 %	35.0 ± 1.9 141 % ***	26.3 ± 1.0 (13) 120 % ***	--
3 x 10^{-8}	38.6 ± 1.2 (9) 145 % ***	1.54 ± 0.14 (7) 87 %	37.1 ± 1.4 149 % ***	30.8 ± 2.0 (9) 141 % ***	50.0 ± 5.7 (6) 151 % **
1 x 10^{-7}	43.3 ± 2.0 (25) 162 % ***	0.97 ± 0.11 (14) 55 % ***	42.3 ± 1.9 170 % ***	33.7 ± 2.2 (25) 154 % ***	53.0 ± 2.6 (13) 160 % ***
1.5 x 10^{-7}	47.5 ± 2.9 (9) 178 % ***	1.08 ± 0.12 (6) 61 % ***	45.7 ± 2.7 184 % ***	35.7 ± 3.3 (9) 163 % ***	58.4 ± 4.4 (9) 176 % ***
2 x 10^{-7}	47.5 ± 2.8 (17) 178 % ***	1.12 ± 0.11 (11) 63 % ***	46.4 ± 2.7 186 % ***	37.0 ± 2.9 (17) 169 % ***	64.2 ± 2.6 (9) 194 % ***
4 x 10^{-7}	50.4 ± 3.5 (14) 189 % ***	1.04 ± 0.16 (7) 59 % ***	49.4 ± 3.3 198 % ***	34.9 ± 2.6 (14) 159 % ***	--
5 x 10^{-7}	45.4 ± 3.4 (12) 170 % ***	0.94 ± 0.05 (12) 53 % ***	44.5 ± 3.0 179 % ***	38.2 ± 2.6 (12) 174 % ***	61.2 ± 4.4 (10) 185 % ***
1 x 10^{-6}	46.5 ± 2.3 (17) 174 % ***	1.07 ± 0.06 (8) 60 % ***	45.4 ± 2.1 182 % ***	39.8 ± 3.4 (16) 182 % ***	63.3 ± 3.8 (8) 191 % ***
EFFECT OF PROINSULIN					
Concⁿ proinsulin (M)					
0	26.8 ± 1.1 (16) 100 %	1.72 ± 0.19 (8) 100 %	25.1 ± 1.3 100 %	22.0 ± 0.7 (16) 100 %	32.0 ± 1.9 (8) 100 %
1 x 10^{-8}	27.0 ± 2.0 (5) 101 %	--	--	23.0 ± 1.9 (6) 104 %	--
1 x 10^{-7}	32.5 ± 2.2 (10) 121 % *	1.28 ± 0.05 (4) 74 % *	31.2 ± 1.9 124 % *	29.4 ± 0.7 (10) 134 % ***	38.2 ± 1.7 (4) 118 % *
2 x 10^{-7}	34.9 ± 1.8 (8) 130 % **	0.97 ± 0.15 (4) 56 % *	33.9 ± 2.0 135 % **	28.0 ± 1.0 (8) 127 % ***	50.3 ± 3.2 (8) 155 % **
4 x 10^{-7}	34.3 ± 3.1 (4) 128 % *	0.92 ± 0.06 (4) 53 % **	33.4 ± 2.8 133 % *	27.6 ± 1.1 (4) 126 % **	--
5 x 10^{-7}	37.2 ± 1.2 (6) 139 % ***	0.91 ± 0.08 (4) 53 % **	36.3 ± 1.2 145 % ***	30.3 ± 1.2 (6) 138 % ***	50.1 ± 4.1 (8) 155 % **
1 x 10^{-6}	37.6 ± 1.7 (4) 140 % ***	0.90 ± 0.09 (3) 52 % *	36.7 ± 1.6 146 % **	29.0 ± 2.2 (3) 132 % *	--

Values are given as means ± SEM. The percentage and Fisher's 'P' values are for the treated animals versus the controls.

the conversion of C6 of glucose to $^{14}CO_2$ is similar to that reported by Greenbaum et al. [5]. The effect of the depressed rate of oxidation of C6 is to increase further the effect of insulin and proinsulin on the C1 - C6 value, the maximal stimulation being, on average, +86% and +46% respectively. The pattern of response with respect to the concentration of the hormone producing half-maximal stimulation is not altered by the opposing effects on C1 and C6 of glucose by these two hormones.

Agius et al. [8] have studied the effect of insulin and proinsulin on the induction of enzymes involved in lipogenesis in hepatocyte cultures. The maximal effects of insulin and proinsulin on glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and 'malic enzyme' were similar. The half-maximal effective concentrations were 3-4 x 10^{-9}M for insulin and approximately 1 x 10^{-7}M for proinsulin. Their values are of the same order of magnitude as those reported here for the half-maximal effect of these hormones in stimulating the oxidation of glucose via the PPP in lactating rat mammary gland slices of 1 x 10^{-8}M for insulin and 1 x 10^{-7}M for proinsulin.

Effect of insulin and proinsulin on lipogenesis from glucose. The addition of insulin in vitro produces a stimulation of +80% in C1 incorporation and +90% in C6 incorporation into lipid, the half-maximal effect being observed at approximately 3 x 10^{-8}M concentration (Table 1). The extent of stimulation and sensitivity to insulin are closely similar to those observed for glucose oxidation via the PPP. This similarity in response is related to the close integration of the two pathways via the rate of utilization of NADPH for lipogenesis and the provision of NADP+ for glucose oxidation via the PPP, a linkage which is particularly marked in lactating rat mammary gland [1,2,5,20]. Again, proinsulin shows a lesser maximal effect on lipogenesis relative to insulin, the extent of stimulation being +32% for C1 and +55% for C6 (Table 1).

Peavy et al. [10] have studied the potency of proinsulin by its ability to stimulate glucose incorporation into lipid in adipocytes. The glucose incorporation into lipid was half-maximal at an insulin concentration of 5 x 10^{-11}M; it required a proinsulin concentration of 1.5 x 10^{-8}M to achieve the same effect. These authors also found that proinsulin had an antilipolytic potency that was less than 1% that of insulin. Comparison of the adipose tissue response with the present data, shown in Table 1, suggests that, while lactating rat mammary gland requires higher concentrations of insulin and proinsulin to achieve half-maximal effects, there is, in contrast, only a 10-fold difference in potency.

Williamson [16] has drawn attention to the importance of the integration of mammary gland metabolism with that of other organs, in particular with liver and adipose tissue. Insulin is a key factor in regulating the metabolism

of the lactating mammary gland and a mechanism must exist to direct anabolic reactions specifically towards the biosynthesis of milk constituents in lactation; prolactin has been postulated to play such a role [see 16].

The question now arises as to whether proinsulin may also be a factor in inter-organ regulation. Such a role would be attractive in the light of the reported increase of proinsulin levels in diabetic pregnant women [14]; parallel data on proinsulin changes in lactation would be valuable.

Some support for a potential role for proinsulin in the differential regulation of adipose tissue and lactating mammary gland glucose metabolism and lipogenesis comes from the present data showing an approximate 10-fold difference in the biological potency, based on half-maximal stimulation of the PPP and lipogenesis (Table 1), while adipocytes have been reported to show a 100-fold difference in sensitivity with respect to lipogenesis from glucose [10].

Further studies on the changes in circulating levels of insulin and proinsulin during the lactation cycle and on the effects of these hormones on preparations of isolated mammary gland cells and adipocytes from the same animals may throw light on the possible importance of proinsulin in the integration of organ metabolism.

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