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THE EFFECT OF ISOPROTERENOL AND β-SYMPATHOMIMETIC DRUGS ON HUMAN PLACENTAL GLYCOGEN METABOLISM

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Abstract—Human placental glycogen metabolism is studied, *in vitro*, by means of three parameters: glycogen level, glycogen phosphorylase and glycogen synthase activities. No significant decrease of glycogen content is observed after incubation of placental fragments with 1×10^{-4}M isoproterenol or salbutamol. In placental homogenates, isoproterenol induces an increase of glycogen phosphorylase a activity and a simultaneous decrease of glycogen synthase a activity. This effect is time and dose-dependent. β sympathomimetic drugs (isoxsuprine, salbutamol) have only a slight action on the activity of both these placental enzymes.

The β sympathomimetic drugs (β mimetics) are largely used for their tocolytic action in obstetrics, to suppress uterine activity, mainly in the management of premature labor [1]. Among their side effects, maternal [2–8] and foetal [9–11] hyperinsulinemia and hyperglycemia have been observed. The aim of this study is to investigate, *in vitro*, the action of β mimetics on the placental glycogen level and on the glycogen phosphorylase (E C. 2.4.1.1) and glycogen synthase (E C. 2.4.1.11) activities. The results are compared to those obtained with a β adrenergic agonist: isoproterenol. We have demonstrated in a previous work [12] that catecholamines increase the placental glycogen phosphorylase activity, the potency order being : isoproterenol > epinephrine > norepinephrine, suggesting a β adrenergic response.

MATERIALS AND METHODS

αD[^{14}C (U)] glucose-1-phosphate (G.1.P), dipotassium salt (sp. act. 294 mCi/mmole) was supplied by NEN, and uridine diphospho-D[^{14}C (U)] -glucose (UDPG), ammonium salt (sp. act. 200 mCi/mmole) by the Radiochemical Centre. Glycogen, from rabbit liver, G.1.P. and UDPG were supplied by Boehringer Mannheim, caffeine by Calbiochem, L-isoproterenol-D-bitartrate and anthrone by Sigma. Other chemicals were purchased from Merck. The β mimetic drugs were generously provided by Mrs. Gardey (Laboratoire de Pharmacologie, Pr Olive, CHU Cochin, Paris).

Human term placentas were obtained from normal pregnancies, immediately after delivery.

For glycogen level estimations, small fragments (20 mg) of placental tissue were incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, for 30 min at 37°, with or without β adrenergic agents. Triplicates were performed for each compound added and for each placenta tested. The tissue glycogen content was measured by the colorimetric method of Seifter *et al.* [13] using anthrone reagent.

For enzymatic determinations, the tissues were rapidly homogenized in ice-cold 5×10^{-2}M Tris–HCl buffer (pH 7.4) in a Potter–Elvehjem apparatus, to give a final tissue concentration of 20 per cent (w/v). Placental homogenate (5 ml) was incubated at 37° with each of the various drugs. At different times of incubation, 3 samples were taken for enzymatic estimations. Samples without drugs served as controls.

Glycogen synthase a activity was measured by the filter paper assay described by Thomas *et al.* [14]. The reaction mixture contained 0.06 μCi^{14}C-UDPG, 5×10^{-3}M UDPG, 0.48% glycogen, 1.7×10^{-3}M EDTA in 4×10^{-2}M Tris–maleate buffer (pH 7.0) and enzymatic preparation in a final volume of 300 μl. For a + b determination we used 1×10^{-3}M UDPG as substrate and we added 3.2×10^{-2}M G.6.P.

Glycogen phosphorylase a activity was tested using the same procedure, modified by Gilboe *et al.* [15] in the presence of 0.05 μCi^{14}C-G.1.P, 2.6 $\times 10^{-2}$M G.1.P, 0.42% glycogen, 0.1 M NaF and 5×10^{-4}M caffeine according to Stalmans and Hers [16] at pH 6.1; 25 μl of enzymatic preparation were added to obtain a final concentration of 100 μl.

Triplicates were performed for each enzymatic estimation, and protein concentrations were determined according to the method of Lowry *et al.* [17] using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Glycogen content was expressed in mg/g wet weight of tissue. In 6 full-term placentas, incubated for 30 min at 37° without addition of effector, the mean value ± S.D. was 1.74 ± 0.19 mg/g. No significant decrease of this glycogen concentration was observed after incubation in the presence of either 1×10^{-4}M isoproterenol (1.67 ± 0.37 mg/g) or

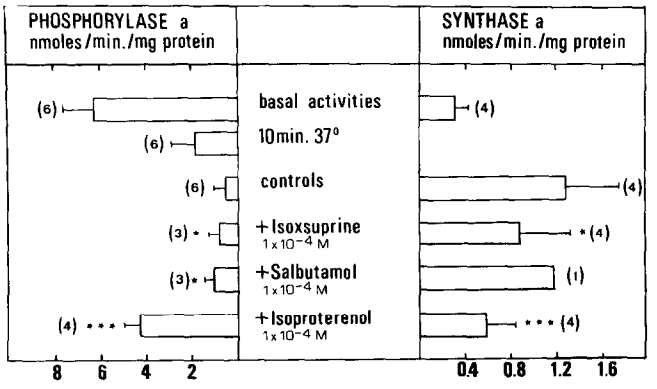

Fig. 1. Action of β sympathomimetic drugs and isoproterenol on glycogen phosphorylase a and glycogen synthase a activities measured in placental homogenates. Bars represent the mean value ± S.D. The significance of differences between the experiments without (controls) or with effectors are tested by application of the unpaired student's *t*-test. * $0.02<P<0.05$. *** $P<0.001$.

5×10^{-4}M salbutamol (1.74 ± 0.22 mg/g). The same mean values were obtained with 1×10^{-3}M, 1×10^{-4}M and 1×10^{-5}M salbutamol. These results are in agreement with our previous work [12] in which glycogen content was assayed by an enzymatic method [18]. By this technique we also obtained the same value in tissue incubated without any drug or after the addition of 1×10^{-4}M isoproterenol or isoxsuprine.

The specific activities of glycogen phosphorylase and synthase were expressed in nmoles of substrate converted per min per mg protein.

Under our experimental conditions, we found that the a form of the glycogen phosphorylase exhibited 2/3 of the total initial activity (a + b) measured in the presence of 9×10^{-3}M 5'AMP. It was therefore essential to inactivate the endogenous glycogen phosphorylase before studying the effect of β adrenergic agents on the reactivation of this enzyme. For this reason the crude placental homogenate was preincubated for 10 min at 37°, without NaF, to convert the active form a into the inactive form b by endogenous phosphorylase phosphatase [19] as seen in Fig. 1. β adrenergic effectors were then added and incubations carried out for 10 min more. Preincubation is not necessary for glycogen synthase a measurement, the basal activity of this enzyme being chiefly in the inactive form. Figure 1 shows that in the controls there is a decrease of glycogen phosphorylase a activity and a simultaneous increase of glycogen synthase a activity after incubation.

In the presence of β mimetic drugs, isoxsuprine (1×10^{-4}M) and salbutamol (1×10^{-4}M), a slightly significant ($0.02<P<0.05$) response was obtained for both glycogen phosphorylase a and glycogen synthase a activities. Salbutamol is somewhat more effective. A highly significant ($P<0.001$) effect of 1×10^{-4}M isoproterenol on the activity of both enzymes was observed: glycogen phosphorylase a increased while glycogen synthase a decreased. However, the initial activities were not restored after addition of isoproterenol. This is probably due to the modification of β adrenergic receptors and to the decrease of intracellular concentration of ATP after homogenization.

The effect of isoproterenol is time-dependent, the maximal values being reached after 10 min at 37° (not shown). Under the same experimental conditions, the action of increasing concentrations of isoproterenol is shown in Fig. 2. The maximal response for phosphorylase a and synthase a activities is obtained with 1×10^{-5} and 1×10^{-6}M isoproterenol, respectively. Otherwise, the effect of isoxsuprine is not dose–response dependent.

Thus, it seems that, under our experimental conditions, isoproterenol induces an increase of phosphorylase and a simultaneous decrease of synthase activities. In spite of these enzymatic activity variations, we could not detect any glycogen breakdown in placental fragments incubated for 30 min at 37°; this lack of response could be due to our short incubation procedures. The effect of isoproterenol

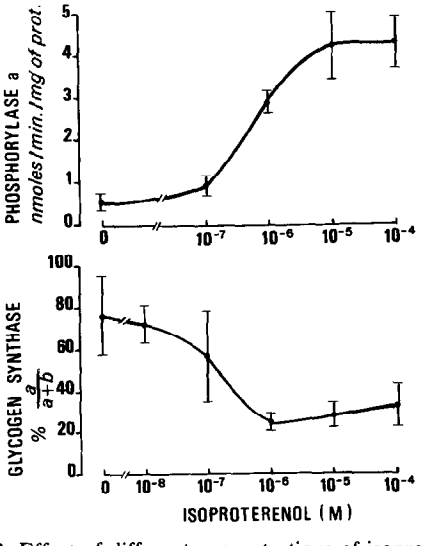

Fig. 2. Effect of different concentrations of isoproterenol on glycogen phosphorylase a and glycogen synthase activities. Bars represent the mean value ± S.D. of 2 experiments with 9 determinations in each case (see Materials and Methods).

can be compared with the stimulating action of another adrenergic compound, epinephrine, on placental adenylate cyclase activity [20]. This action of epinephrine can be antagonized by a β adrenergic blocking agent, propranolol, but not by an α adrenergic blocking agent, phentolamine [21].

By contrast, β sympathomimetic drugs (isoxsuprine, salbutamol) have only a slight action *in vitro* on the activities of the enzymes able to regulate the glycogen metabolism. Yet, *in vivo*, local glycogenolytic action cannot be excluded. However, it is not sufficient by itself to account for the foetal metabolic modifications observed. These modifications might occur by a direct effect on the foetus, considering that the placental transfer of these drugs is possible. It has been shown that 38 per cent of salbutamol is transfered to the foetus [22].

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