Development of Insulin Resistance in Experimental Animals during Long-Term Glucocorticoid Treatment

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 4, pp. 394-396, April, 2002
Original article submitted January 22, 2002

Long-term treatment with glucocorticoids led to the development of insulin resistance in experimental animals, which was confirmed by a progressive increase in blood insulin level and decrease in the glucose/insulin index. The comparative study showed that hydrocortisone produced more pronounced and rapid changes than dexamethasone. However, we found no differences in the glucose/insulin index by the 23rd day of treatment with these hormones.

Key Words: *glucocorticoids; insulin; glucose; glycogen; insulin resistance*

Glucocorticoids are widely used in transplantology and therapy of allergic and hematological disorders, renal, intestinal, liver, eye, and skin diseases. Rheumatic diseases and bronchial asthma are the main indication for long-term therapy with these hormones [5]. However, glucocorticoids often cause side effects [3, 6], in particular, non-insulin-dependent diabetes mellitus. The development of insulin resistance in peripheral tissues induced by glucocorticoids is the major pathogenetic mechanism of this disease [8]. Much research was devoted to evaluation of the mechanisms underlying the development of insulin resistance after administration of dexamethasone for several days (2-3 days) [7]. This experimental model is also used to estimate properties of various preparations [1]. The phenomenon of insulin resistance and dynamics of changes in the sensitivity of tissues to insulin after long-term treatment with glucocorticoids are poorly understood. Previous studies showed that after long-term administration of hydrocortisone, liver cells do not respond to this hormone by induction of gluconeogenesis enzymes [4].

Here we studied the development of insulin resistance in experimental animals after long-term treatment with hydrocortisone and dexamethasone that belong to hormones with short-lasting and long-lasting biological activities [5].

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MATERIALS AND METHODS

Experiments were performed on 64 adult male Wistar rats kept in a vivarium with free access to food and water. Group 1 animals (n=34) received 100 μg/kg dexamethasone intraperitoneally. Blood glucose and immunoreactive insulin (IRI) and liver glycogen content were measured 0.5, 2, 3, and 5 h after single injection of dexamethasone or 5 h after injection on days 10, 15, and 23 of daily treatment with this hormone. Group 2 rats (n=15) received 50 mg/kg hydrocortisone. Test parameters were estimated 5 h after injection on days 1, 10, 15, and 23 of daily treatment with this hormone. Control animals (n=15) were intraperitoneally injected with physiological saline 5 h before euthanasia. Hydrocortisone and dexamethasone in these doses are equally potent in activating liver gluconeogenesis enzymes in animals [4].

Blood glucose concentration and liver glycogen content were measured colorimetrically using *o*-toluidine and anthrone reagents. Plasma insulin was measured by radioimmunoassay using RIO-INS-^{125}I-M kits.

The results were analyzed by the methods of variational statistics. Intergroup differences were analyzed using the Newman—Keuls test. The differences were significant at $p<0.05$.

0007-4888/02/1334-0339$27.00

RESULTS

Blood glucose tended to increase 2 and 3 h after single injection of dexamethasone. Blood concentration of IRI increased 30 min after dexamethasone administration (Fig. 1, *a*). Intensification of glucose utilization in the liver due to the release of additional insulin into the blood was manifested in a 1.6-fold increase in liver glycogen content 5 h after single administration of dexamethasone (Table 1). Previous studies showed that the increase in glycogen content is proportional to the sensitivity of tissues to insulin [2].

After long-term treatment with dexamethasone blood glucose level remained unchanged, while insulin content increased. Blood insulin concentration significantly differed from the control 23 days after the start of daily treatment with the hormone (Fig. 1, *b*).

As differentiated from single injection of dexamethasone, long-term treatment with the hormone was not accompanied by changes in liver glycogen content (Table 1). This indicated the development of insulin resistance in liver cells. The glucose/insulin index reflecting the degree of insulin resistance of peripheral tissues decreased by 1.8 times on days 10 and 15 of dexamethasone administration. On day 23 this parameter was 3.5-fold below the control (Table 1).

Blood glucose and IRI contents surpassed the control starting from day 10 of hydrocortisone treatment (Fig. 1, *c*). It should be emphasized that long-term treatment with hydrocortisone more significantly increased the content of IRI compared to dexamethasone (by 1.5-2.0 times). The glucose/insulin index decreased by 4 times on day 23 of hydrocortisone administration (Table 1).

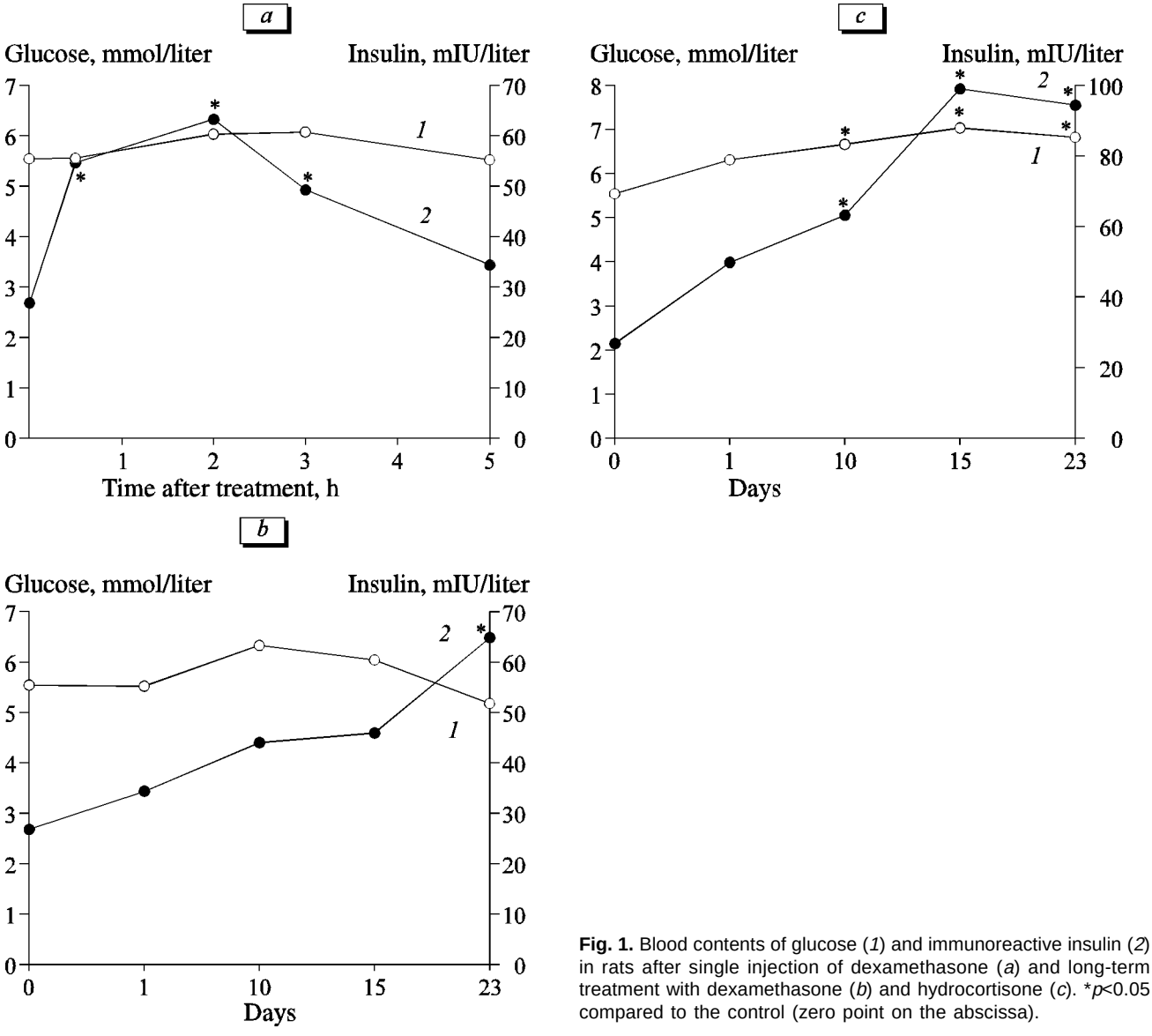

Fig. 1. Blood contents of glucose (*1*) and immunoreactive insulin (*2*) in rats after single injection of dexamethasone (*a*) and long-term treatment with dexamethasone (*b*) and hydrocortisone (*c*). **p*<0.05 compared to the control (zero point on the abscissa).

TABLE 1. Liver Glycogen Content (g/100 g) and Glucose/Insulin Index in Rats Receiving Dexamethasone or Hydrocortisone ($M \pm m$)

Experimental conditions	Glycogen	Glucose/insulin
Control	4.39±0.29	0.28±0.04
Dexamethasone		
30 min	3.69±0.74	0.12±0.04
2 h	4.53±0.96	0.09±0.01*
3 h	4.63±0.85	0.13±0.01
5 h	6.86±0.26*	0.17±0.02
10 days	5.29±0.45	0.15±0.02
15 days	4.19±0.59	0.16±0.04
23 days	5.52±0.34	0.08±0.01*
Hydrocortisone		
1 day	5.11±0.64+	0.13±0.02
10 days	5.79±0.91	0.11±0.01
15 days	3.29±0.58	0.07±0.01*
23 days	4.30±0.83	0.07±0.01*

Note. $p<0.05$: *compared to the control, +compared to dexamethasone.

Despite high insulin concentration in the blood, the liver content of glycogen did not increase, which attested to resistance of liver cells to inducing effect of hydrocortisone.

Our results show that the severity of insulin resistance directly depends on the duration of glucocorticoid treatment. Despite different dynamics of the studied parameters during long-term administration of dexamethasone and hydrocortisone, we found no differences in the severity of insulin resistance of tissues (evaluated by the glucose/insulin index) on day 23 of treatment with these hormones.

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