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Modification of Glucagon-Induced Hyperglycemia in Rats by 17-ethyl-19-nortestosterone. (26498)

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Considerable data are available indicating that 17-ethyl-19-nortestosterone (Nilevar) possesses potent myotrophic and anabolic properties as well as demonstrating a high anabolic to androgenic ratio(1,2,3). Recently, Nilevar was demonstrated to reduce the hyperglycemia associated with injection of glucagon (HGF) in human subjects(4). The specific mechanism by which Nilevar exerts its suppression of a glucagon-induced hyperglycemia is unknown. Blockade of glycogenolytic pathways, decreased gluconeogenesis, mediation through the pituitary or direct action on target endocrine tissues which affect any of the former, or a combination of the foregoing can be cited as the most likely possibilities.

Reported herein are studies carried out to determine more precisely the nature of Nilevar's reduction of glucagon-induced hyperglycemia. The ability of Nilevar to modify the glucagon tolerance test was compared with that of testosterone; additionally, castrated rats were compared with intact animals in their response to the hyperglycemic factor. Finally, hypophysectomized animals pretreated with Nilevar were subsequently injected with glucagon to weigh the possible influence of hypophyseal secretions on the mode of action of Nilevar as an anti-hyperglycemic agent.

Methods. All rats employed in these studies were male descendants of the Sprague-Dawley strain, were fed Purina laboratory chow *ad libitum*, and were kept at a room temperature of 74-78°F. Rats, hypophysectomized at a body weight of 200-210 g, were purchased from the Charles River Breeding Laboratories and were used 20 days postoperatively. All injections were given daily at 9:00 a.m. for 5 consecutive days; the terminal injection was given 2 hours before taking the first blood sample. Both Nilevar and testosterone† were suspended in peanut oil such that 0.15 mg of either steroid was contained in 0.25 ml of vehicle. Control rats received peanut oil only in a volume equal to experimental groups. Cortisone acetate was suspended in saline such that 2.5 mg were contained in 0.25 ml.

The glucagon tolerance test (GTT) was performed on rats fasted 4 hours (except in the study with hypophysectomized rats which were fed *ad libitum* to insure adequate liver

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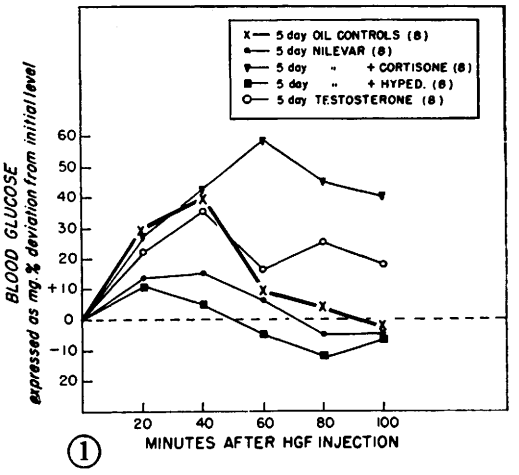

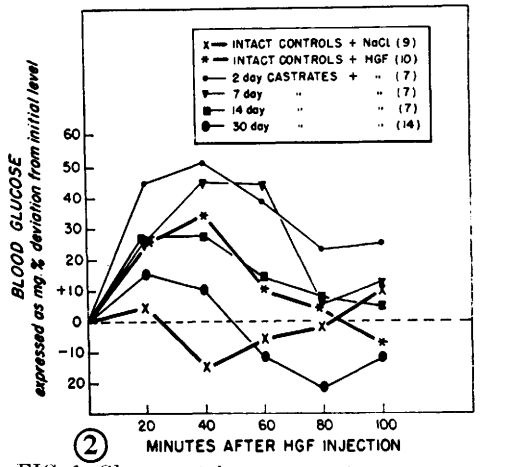

FIG. 1. Glucagon tolerance tests in male rats inj. daily with 0.15 mg testosterone, with 0.15 mg Nilevar, or with 2.5 mg cortisone for 5 consecutive days. All groups were fasted 4 hr except the hypophysectomized rats which were fed *ad libitum*.

FIG. 2. Glucagon tolerance tests conducted in castrated male rats at varying post-operative periods.

glycogen) at the conclusion of which the animal was anesthetized with sodium pentobarbital (40 mg/kg body weight) and a midline ventral incision made. In some cases a very small piece of liver was taken for initial glycogen content. Serial blood samples (0.15 ml each) were obtained from the vena cava below the entrance of the renal vein. The control blood samples were taken immediately after injection of the crystalline glucagon solution‡ (0.1 mg/kg body weight) into the vena cava and subsequent rinsing of the syringe with caval blood. Immediately after the final caval blood sample was drawn a terminal sample was taken from the abdominal aorta with the aid of a heparinized syringe to determine inorganic phosphorus and urea concentrations. Slices of liver and of the right testis were then quickly placed in tared test tubes containing 30% KOH for glycogen determination. The time from drawing the terminal aortic blood sample to the time the tissues were placed in KOH did not exceed 30 seconds.

Total reducing substances were determined on the serial caval blood samples using Nelson's colorimetric adaptation of the Somogyi method(5); serum inorganic phosphorus by the method of Sumner(6); urea by the diacetyl monoxine method of Rosenthal(7); and glycogen analyses were made by the anthrone method of Seifter *et al.*(8).

Results. Series I. Effect of Nilevar, testosterone or cortisone pre-administration on glucagon-induced hyperglycemia. The results are presented in Fig. 1 and Table I and clearly show that intact rats given a glucagon tolerance test (GTT) after a period of 5-daily Nilevar injections demonstrate a modified hyperglycemic response. Additionally, the anabolic steroid lowered blood urea levels significantly ($p<.01$). The presence of the pituitary gland was not essential for Nilevar to exert its anti-hyperglycemic effect as there were statistically significant ($p<.01$) differences between the blood glucose data of this group and those of the oil controls during the established peak of glucagon activity. Despite this anti-hyperglycemic effect, Nilevar was far less effective in increasing the body weight of the operated rats as compared with intact rats; also, there was no evidence of increased liver glycogen or decreased urea levels in the hypophysectomized animals as was seen in the intact rats receiving Nilevar.

Five daily injections of testosterone (at a dose identical with that of Nilevar) failed to modify the GTT in the intact rat. No significant differences were observed when blood glucose data of the oil control group were

‡ Generously donated by Dr. W. R. Kirtley, Eli Lilly Co., Indianapolis, Ind.

TABLE I. Effect of a Single Injection of Glucagon on Blood and Tissue Constituents of Rats Injected Daily for 5 Days with Nilevar, Testosterone and Cortisone.

Group	No. rats	Avg wt gain, g	Blood values in mg % Phosphorus	Urea	Glycogen as mg/g tissue Liver	Testis
Oil controls	8	+20	8.50 ± 1.1*	44.7 ± 3.8	13.6 ± 4.2†	2.14 ± .06
Nilevar (5 days)	8	+38	7.10 ± .1	28.9 ± 1.6	24.9 ± 3.2	2.44 ± .04
Testosterone (5 days)	8	+ 6	7.77 ± .5	38.9 ± 1.0	7.8 ± 1.2	2.16 ± .04
Nil. + cort. (")	8	- 8	6.67 ± 1.2	51.5 ± 1.2	60.2 ± 5.4	2.22 ± .03
Hypox + oil (")	5	0	4.93 ± .3	65.6 ± 2.3	24.4 ± 5.1	2.53 ± .12
" + Nil.(")	8	+ 3	6.51 ± .3	71.1 ± 3.1	5.2 ± .4	2.36 ± .06

* ± stand. error.

† Pre-glucagon liver glycogen levels averaged 46.4 mg/g tissue for oil, Nilevar and testosterone groups; 80.2 mg/g for cortisone and Nilevar group; and 36.2 mg/g for hypophysectomized groups, both of which were non-fasted.

compared with those of the testosterone group. Far less increment in body weight occurred with testosterone than when either oil alone or Nilevar was injected, and blood urea, inorganic phosphorus and testicular glycogen appeared essentially unaltered (Table I).

When a daily dose of 2.5 mg of cortisone acetate was injected along with the standard dose of 0.15 mg Nilevar for 5 days, a supernormal response to the GTT lasting beyond the 100 minute observation period was observed. Also, in addition to this hypersensitivity to exogenous glucagon, blood urea and liver glycogen analyses indicate the failure of 0.15 mg Nilevar daily to suppress the powerful gluconeogenic action of the glucocorticoid.

Series II. Sensitivity of castrated male rats to exogenous glucagon. To test whether rats deprived of their normal endogenous supply of anabolic-androgenic secretions would demonstrate any altered response to the pancreatic hyperglycemic factor, groups of rats were castrated and subjected to a GTT at varying post-operative times. Hypersensitivity to exogenous glucagon is apparent in Fig. 2 where rats castrated 2 or 7 days previous to the GTT can be compared with intact controls ($p<.01$ at every point for the 2-day castrate group; $p<.01$ at the 40 and 60 min points for the 7-day castrate group). That this hypersensitivity to glucagon is transitory is apparent from the normal GTT response of the 2-week castrate group and by 30 days post-operatively there appears to be a definite resistance to HGF ($p<.05$, $<.01$,$<.01$ for the 40, 60 and 80 min blood samples respectively). Urea levels (Table II) increased significantly when compared with intact control values ($p<.01$) as early as 2 days post-operatively, returned to normal by the seventh post-operative day, only to rise once again by the thirtieth post-operative day ($p<.01$).

Discussion. As in man(4), Nilevar is an effective agent in reducing the hyperglycemic response evoked by exogenous glucagon in rats. Testosterone, however, appears to be without effect in this respect. It is unlikely that Nilevar-induced resistance to glucagon is mediated *via* hypophyseal activity, or even through the hypophyseal target areas, since the rats employed in these studies were hypophysectomized 20 days previously. Pro-

TABLE II. Effect of a Single Injection of Glucagon on Serum Inorganic Phosphorus, Urea, and Liver Glycogen in Castrated Male Rats.

Group	No. rats	Body wt, g	Blood values in mg % Phosphorus	Urea	Glycogen in mg/g tissue Liver	Testis
Intact controls + NaCl	9	183 ± 9.1*	7.65 ± .7	33.6 ± 2.6	46.3 ± 5.9†	2.35 ± .06
" " + HGF	10	184 ± 6.9	8.62 ± 2.9	37.5 ± .7	11.8 ± 3.4	3.23 ± 1.8
2 days castrate + HGF	7	161 ± 7.4	7.82 ± .4	52.8 ± 1.9	16.7 ± 3.1	—
7 " *idem*	7	180 ± 2.4	8.64 ± .7	39.0 ± 1.5	9.0 ± 2.5	—
14 " "	7	251 ± 5.3	7.95 ± .8	38.9 ± 2.3	19.6 ± 1.8	—
30 " "	14	313 ± 4.6	7.41 ± .2	48.2 ± 1.3	17.7 ± 3.3	—

* ± stand. error.

† Pre-glucagon liver glycogen levels were in range of 38.5-49.1 mg/g tissue for all groups.

gressive postoperative atrophy of these tissues should render such an action partially or totally ineffective(9). Even though the GTT was modified in these operated animals, liver glycogen was grossly depleted indicating that blockade of glycogenolysis was not the mode of action of Nilevar in these preparations. That the protection against glucagon-induced hyperglycemia afforded by Nilevar may be mediated through gluconeogenic channels was evidenced by the sparing of protein concomitant with liver glycogen storage in the groups receiving Nilevar alone, as well as the exaggerated GTT curves, liver glycogen and urea levels in rats injected simultaneously with a gluconeogenic steroid such as cortisone. The hypersensitivity of the cortisone-injected rats to glucagon is in agreement with data reported on other species subjected to this double-hormone treatment(10).

Additional information as to the possible mechanism of action of Nilevar in curtailing the GTT was gained by testing the glucagon sensitivity of castrated male rats. The increased sensitivity of castrated rats to the hyperglycemic activity of HGF was transitory, the 2 and 7 days post-operative groups being hypersensitive, the 14-day castrates normal in their response, and 30-day postoperative group being refractive to exogenous glucagon. The explanation for the resistance which the one month castrate group demonstrated to glucagon is not readily available and is complicated by the fact that these animals paralleled the hypersensitive 2-day castrates in all other parameters studied (Table II). The early post-operative hypersensitivity to glucagon, in contrast to the ineffectiveness of injected testosterone to modify the GTT, would indicate that the mechanism whereby Nilevar exerts its protective influence is probably not mediated by a simple regulation of endogenous testosterone secretion.

The testicular glycogen data presented herein emphasize the stability of this carbohydrate moiety and are in accord with observations of other workers(11). Thus, a small but definite level of this polysaccharide exists in the rat testis and it must be concluded that it is of little importance in contributing to the hyperglycemia induced by exogenous glucagon.

Summary. Nilevar, but not testosterone, was effective in reducing the hyperglycemic response to exogenous glucagon in rats. Castrated rats were hypersensitive to the glucose elevating properties of glucagon for several days immediately after operation but by 30 days were resistant to the effects of glucagon. The refractory state induced by Nilevar probably was not mediated through the hypophysis, the hypophyseal target glands, or by blocking hepatic glycogenolytic pathways.

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