Stimulatory Effects of Insulin and Glucagon on Phenylethanolamine-*N*-Methyl Transferase of Rat Adrenal* (32676)

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Phenylethanolamine-*N*-methyl transferase (PNMT) is an enzyme which is highly localized in the adrenal medulla and catalyzes the conversion of norepinephrine to epinephrine. The activity of this enzyme is decreased in hypophysectomized fetal or adult rats (1,2) but is partially corrected by the administration of ACTH or glucocorticoids (1).

The injection of insulin into dogs is associated with an increase in release of epinephrine and norepinephrine from the adrenal vein with a greater release of epinephrine than norpinephrine (3). One explanation for this phenomenon could be that insulin stimulates PNMT. Indeed, preliminary results from this laboratory indicated that insulin and glucagon each stimulates PNMT activity (4, 5). The present paper reports more extensive confirmation of these conclusions.

Methods and Results. Female Wistar rats of different weights were given 10% glucose in water for 3 days, and various test agents were given intraperitoneally at different intervals. The animals were sacrificed and blood was collected for the determination in serum of glucose by the Technicon Autoanalyzer ferricyanide method and of insulin by the double-antibody technique of Morgan and Lazarow (6). The adrenals were removed, cleaned of adhering fat, weighed, and homogenized in 2.5 ml of isotonic KCl. The homogenate was centrifuged in a model-L Spinco Ultracentrifuge at 100,000 *g* for 30 min. The supernatant solution was collected for enzyme assay, using the method of Wurtzman and Axelrod (1). The enzyme activity is reported as units per pair of adrenal. One unit of enzyme is defined as 1 mμmole of metanephrine formed in 60 min from normetanephrine.

The enzyme preparations from the control and test samples were always assayed on the same day to minimize fluctuations due to day to day assay variation. With the exception of the experiments on Table III, all enzyme assays were performed immediately after the preparation of the enzyme. The enzyme assays on Table III were done on the preparations which were frozen for 48 hours. Although freezing and thawing cause some loss of enzyme activity, the difference between the control and insulin injection remains unaltered in the fresh or the frozen preparations (unpublished observations).

The results in Table I show that when the rats were given 1,2,3,4,5, and 6 units of lente insulin, respectively, on the first, second, third, fourth, fifth, and sixth days, and were then sacrificed 24 hours after the last dose of insulin; the PNMT activity was higher than that of the saline-injected controls. This table also compares the adrenal weight and serum glucose in both groups. Insulin injection stimulated the enzyme activity by 28% which was highly significant. The serum glucose from the insulin-treated rats as determined at the time of sacrifice was not decreased. To determine the earliest time necessary for the insulin effect on PNMT activity, two groups of rats were injected with 6 units of regular insulin 1 hour and 6 hours prior to sacrifice. The results show (Table II) that although the reduction of serum glucose occurred during the first hour of insulin injection, no significant change in enzyme activity occurred. Whereas 6 hours after the insulin injection, a 10% stimulation of the enzyme activity occurred which was significantly different from the control group. Table III illustrates the result of a similar experiment with lente insulin. The stimulatory effect of lente insulin, however, was not significant until 24 hours after the injection of 6 units. Although serum glucose did not appear to be decreased at the time of sacrifice,

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TABLE I. The *in Vivo* Effects of Lente Insulin on PNMT Activity.[a]

Treatment	Body weight (gm)	Adrenal weight (mg)	Serum glucose (mg/100 ml)	IRI[b] (μU/ml)	PNMT (% activity)
Control	169 ± 9	42.3 ± 0.7	138 ± 1	26 ± 2	100
Insulin injected (see text)	172 ± 9	45.9 ± 0.8	165 ± 1	35 ± 6	128[c]

[a] Total of 13 pairs of rats were used for the enzyme assay. Incubation system: 50 μl of adrenal supernatant fluid, 37.5 μg of DL-normetanephrine–HCl, 1 mμmole of ^{14}C-S adenosylmethionine, and 100 μmoles of phosphate buffer, pH 7.9, in a total volume of 250 μl.

[b] Immunoreactive insulin in four pairs of rats.

[c] $p < .005$.

TABLE II. Effect of Crystalline Insulin on PNMT Activity of the Rat Adrenal.

Treatment	Body weight (gm)	No. of rats	Adrenal weight (mg)	Serum glucose (mg/100 ml)	IRI[a] (μU/ml)	PNMT	p
Control	213 ± 9	3	52.0 ± 6.0	153 ±13	27.7 ± 0.3	2.69 ± 0.05	
After 1 hour of insulin	220 ±10	3	57.0 ± 3.0	78 ± 9	424 ±27.7	2.81 ± 0.45	NS
Control	131 ± 2	6	28.8 ± 0.9	141 ± 2	47 ± 2.9	1.75 ± 0.05	
After 6 hours of insulin	128 ± 1	6	26.6 ± 0.8	139 ± 2	65 ± 8.4	1.99 ± 0.06	<.02

[a] Immunoreactive insulin ± SEM.

TABLE III. Effect of Lente Insulin on PNMT Activity of the Rat Adrenal.

Treatment	Body weight (gm)	No. of rats	Adrenal weight (mg)	Serum glucose (mg/100 ml)	PNMT
Control	162 ± 2[a]	3	38.3 ± 2.7	150 ± 3	1.21 ± 0.10
After 12 hours of insulin	158 ± 3	6	36.0 ± 5.0	154 ± 5	1.26 ± 0.17
After 24 hours of insulin	161 ± 2	4	41.0 ± 5.4	162 ± 9	1.63 ± 0.17[b]

[a] ± SEM.

[b] Differs from control group ($p < .002$).

it was possible that transient hypoglycemia periods had occurred earlier. To study this phenomenon, groups of rats were given regular and lente insulin, as well as glucagon, and were then sacrificed at various times to determine changes in serum glucose. Figure 1 depicts the results of these experiments. Profound hypoglycemia occurred with regular and lente insulin during the first 3 hours. The serum glucose, however, gradually returned to normal levels in 4–6 hours. In contrast, zinc glucagon caused a transient hyperglycemic effect during the first hour with prompt return of the serum glucose to normal levels in 2 hours. Since glucagon is a known stimulator of insulin secretion (7) and epinephrine release (8,9), independent of hypoglycemia, the effect of glucagon on PNMT activity was studied. With injection of 1 mg of zinc glucagon there was a slight but significant stimulation of PNMT activity with 6 hours after glucagon injection (Table IV). Since diazoxide inhibits insulin secretion (10), the effect of 1.5 mg of intraperitoneal injection of diazoxide into six rats was studied (Table IV). Diazoxide in this dosage failed to stimulate PNMT activity significantly.

Since hypophysectomy is known to decrease

TABLE IV. Effect of Glucagon and Diazoxide on PNMT Activity of the Rat Adrenal.

Treatment	Body weight (gm)	No. of rats	Adrenal weight (mg)	Serum glucose (mg/100 ml)	IRI[a] (μU/ml)	PNMT
Glucagon (6 hours)[c]	126 ± 3	6	28.7 ± 0.2	147 ± 5	82 ±19.1	2.03 ± 0.09[b]
Control	131 ± 2	6	28.8 ± 0.9	141 ± 2	47 ± 2.9	1.75 ± 0.05
Diazoxide (3 hours)[c]	128 ± 1	6	29.5 ± 1.4	147 ± 1	38 ± 4.4	1.84 ± 0.11

[a] Immunoreactive insulin.

[b] ± SEM. Differs from control group ($p<.05$).

[c] Refers to periods after the injections.

PNMT activity (1), the effect of insulin alone, and in conjunction with ACTH, was studied in hypophysectomized rats (Fig. 2). The results confirmed the findings of Wurtman and Axelrod (1) that hypophysectomy decreases enzyme activity. Furthermore, ACTH injection (4 units daily for 6 days) restored enzyme activity to about 60% of the normal value but insulin and ACTH in combination brought the enzyme activity to its original level. Insulin alone had no effect in stimulating PNMT activity in hypophysectomized rats.

The enzyme activity in Fig. 2 is reported per pair of the adrenal glands [as also reported by Wurtman and Axelrod (1)]. Although other means of reporting enzyme activity (such as per mg of adrenal weight, per mg of protein, or per 100 gm of body weight) may alter our conclusion in Fig. 2, there are no published studies which suggest that, in the crude enzyme preparation, total body weight, or total adrenal weight are determining factors of enzyme activity. In fact, in metyrapone-treated rats where adrenal cortex hypertrophy occurs (1), although enzyme activity per pair of adrenals remains the same, the expression of enzyme activity on the basis of milligrams of adrenal or milligrams of protein would have been misleading.

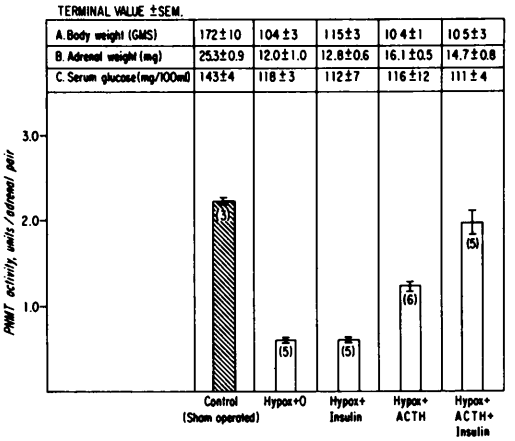

FIG. 2. Effect of hypophysectomy, insulin, and ACTH injections on phenylethanolamine-*N*-methyltransferase (PNMT) of rat adrenal. Animals were sham-operated or hypophysectomized, and killed 1 week later. Hypox + 0, hypophysectomized without treatment. Hypox + Insulin, hypophysectomized and received total of 20 units of lente insulin over 6-day period. Hypox + ACTH, hypophysectomized and received 4 units of ACTH gel subcutaneously daily for 6 days. Hypox + ACTH + Insulin, hypophysectomy plus combination of above doses of insulin and ACTH.

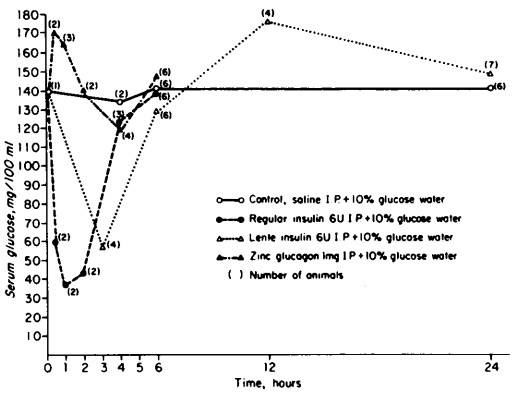

FIG. 1. Effect of regular insulin, lente insulin, and zinc glucagon on serum glucose of the rat at various times after intraperitoneal injection of above agents.

The mechanism of action of glucagon and insulin was studied as shown in Table V. In this experiment, groups of rats were given simultaneous injections of 6 units of crystalline insulin and 1 mg of zinc glucagon and were then sacrificed at various times follow-

TABLE V. Effect of Zinc Glucagon and Crystalline Insulin on PNMT Activity of the Rat Adrenal.

Hours after injection	Body weight (gm)	No. of rats	Adrenal (mg)	Serum glucose (mg/100 ml)	PNMT
Control	139 ± 2[a]	5	33.4 ± 1.0	144 ± 3	1.86 ± 0.18
2	130 ± 1	5	28.8 ± 2.0	93 ± 24	2.79 ± 0.19[b]
4	133 ± 3	5	30.4 ± 1.9	86 ± 19	2.55 ± 0.32
6	127 ± 1	5	31.8 ± 1.8	67 ± 17	1.96 ± 0.22
10	131 ± 3	5	34.0 ± 1.5	103 ± 24	2.23 ± 0.27
24	133 ± 3	5	32.0 ± 1.6	132 ± 9	1.77 ± 0.35

[a] ± SEM.

[b] Differs from control ($p < .01$).

ing the treatment. Serum glucose, adrenal weights, body weights, and PNMT activity were compared in all the groups. As shown in this table, glucagon and crystalline insulin exerted a significant (49%) stimulatory effect on PNMT activity only during the first 2 hours after injection, with a suggestion (statistically insignificant) of slight stimulation for 2 additional hours. The PNMT activity returned to the control value 6, 10, or 24 hours after injection of the agents.

Comparison of Tables III and IV reveals that the stimulatory effect of *lente* insulin on PNMT activity is much more delayed (Table III) than that of *crystalline* insulin and zinc glucagon (Table V) where a shorter duration of action is noted.

The *in vitro* addition of insulin, ACTH, or hydrocortisone to the enzyme preparation of the control animals showed no effect on the enzyme activity.

Discussion. Although the regulation of PNMT activity by glucocorticoids as suggested by Wurtman and Axelrod (1) is intriguing, this regulation does not seem to be an adequate explanation for controlling the enzyme activity, since (1) the endogenous glucocorticoids in an intact rat exert no further stimulatory effect on PNMT activity (11), and (2) selective inhibition of glucocorticoids by metyrapone does not alter enzyme activity (1). Furthermore, in hypophysectomized rats, addition of ACTH only partially restores the PNMT activity. Although the mechanism by which insulin stimulates PNMT activity is not clear, one suggestion is that insulin injection stimulates glucocorticoid production through hypoglycemia (12). However, increased glucocorticoid production in the intact rat has been shown to be ineffective in stimulating PNMT activity (11). Furthermore, the injection of glucagon, which has no apparent hypoglycemic effect in rats, results in significant stimulation of this enzyme activity. It is clear that for maximal enzyme stimulation, both glucagon and insulin are needed. One explanation for this phenomenon may be that glucagon, through endogenous insulin stimulation, further augments the effect of insulin.

Insulin has been shown to stimulate the key enzymes in the glycolytic pathway (13). Whether insulin is also an inducer of PNMT enzyme is suggested but not proved by the present work.

Although the physiological significance of insulin on PNMT is not established, it is tempting to postulate that insulin in the intact rat may partially exert some controlling influences on elaboration of epinephrine in the adrenal medulla.

Thus, while increase in level of insulin might stimulate production of epinephrine, the increased concentration of the latter substance may exert an end product inhibition on PNMT activity (11) as well as insulin release (14).

Summary. Injection of glucagon and insulin in rats stimulates the adrenal medullary enzyme, phenylethanolamine-*N*-methyl transferase (PNMT), with evidence of this being apparent within 2 hours. The highest level of stimulation results when glucagon (1 mg) and insulin (6 units) are injected simultaneously.

In hypophysectomized rats, there is a marked decrease in the enzyme activity. ACTH only partially corrects this, but insulin *and* ACTH increase the enzyme activity to normal levels. It is suggested that, in the intact rats, insulin and glucagon may exert a stimulatory effect on PNMT activity independent of hypoglycemia or increased glucocorticoid production.

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Composition of Hypertrophic and Atrophic Muscles in Genetic Muscular Dystrophy of the Chicken* (32677)

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Hereditary muscular dystrophy in the chicken affects primarily the muscles which normally contain a high proportion of white fibers. The dark or predominantly red muscles are involved to a much lesser degree (1) Similar observations on dystrophic mice have been made by Brust (2) in comparing characteristics of the soleus and gastrocnemius. In dystrophic chickens the white pectoralis muscle has been most studied because of size, accessibility to biopsy, and invariable involvement in dystrophic processes. Hypertrophy of the pectoralis is observed in all dystrophic chickens at an early age. In some birds this is followed by gross atrophy with accompanying fatty infiltration while in others hypertrophy may persist to an advanced age. Asmundson has selected lines of dystrophic chickens which exhibit either early atrophy (line 307) or persistent hypertrophy (lines 304 and 308) (3).

Dystrophic pectoralis muscle when compared with normal pectoralis contains considerably more fat, which may obscure the true relationships of the tissue components if they are merely estimated on a wet-weight basis. It has long been known that in the development of the embryo the water content of the tissues gradually decreases and the nitrogen content gradually increases with age. After birth or hatching this process continues until at the end of the juvenile phase of growth the total body and the muscles reach an ap-

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