Adrenal Catecholamine Levels and Synthesizing Enzyme Activities in Newborn Swine Exposed to Cold and 6-hydroxydopamine (36917)

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The *in vitro* activities of tyrosine hydroxylase (TH) and/or phenethanolamine *N*-methyltransferase (PNMT) from the adrenal glands of mature rodents have been reported to be increased by the following treatments: 1. administration of reserpine, phentolamine or 6-hydroxydopamine, 2. repeated immobilization, 3. prolonged exposure to severe cold and 4. electrical stimulation of the hypothalamus. The increases in activity are apparently due to the synthesis of new enzyme, which is induced when reflex, sympathetic nervous system tone is increased (1). Leduc (2) reported that adrenal catecholamine content was depleted in mature rats exposed to severe cold. However, enhanced synthesis soon replenished the hormone levels in the gland. The effects of stress on the function of the adrenal medulla do not seem to have been studied in the neonatal swine, even though the levels of total catecholamines and the percent of norepinephrine in the gland of this species are high compared to most mammals (3).

We have, therefore, investigated the effects of cold stress, handling stress and the administration of 6-hydroxydopamine on adrenal catecholamine levels and the activities of TH or PNMT in the gland. This work is part of a continuing program in our laboratory to identify biochemical or physiological deficiencies in the newborn swine which may account for the high mortality rate (20–30%) of the live born of this species during the first few days of life (4).

Materials and Methods. Animals and handling. The Chester White piglets were farrowed in temperature-controlled facilities. The newborn animals were allowed to nurse once before they were used. The animals to be exposed to cold or warm were transported from the farrowing barn to the laboratory (a distance of about 200 yards) in an air conditioned vehicle and maintained at thermoneutrality (34°) for 30 min. Animals to be exposed to cold were placed in metal containers in a cold room maintained at 5°. Control animals were maintained at 34° with heat lamps under similar conditions. The piglets which were treated with 6-hydroxydopamine or 0.9% NaCl solution were taken from the sow, injected and immediately returned to the litter. At the time of sacrifice, the animals were placed in small containers which were then covered with a plexiglass lid with air intakes. Cold-stress animals and their controls were anesthetized directly in the exposure chambers without additional handling. Halothane anesthesia was introduced into the chambers by pumping room air through a Fluotec 3™ Vaporizer (Fraser Sweatman, Inc., Hatfield, Pa.) connected to a small vacuum/pressure pump. The adrenal glands of the anesthetized piglets were removed, trimmed, frozen in liquid nitrogen and stored at −20° until they were assayed. (The levels of porcine adrenal gland catecholamine and the activities of tyrosine hydroxylase and phenethanolamine *N*-methyltransferase did not change significantly in tissue stored at −20° for at least 14 days.)

Biochemical analyses. The catecholamines contained in 0.4 *M* perchloric acid extracts of whole adrenal glands were absorbed on the weakly acidic carboxylic acid cation-exchange resin, BioRex 70 (BioRad Lab., Richmond, Calif.) (5). Trihydroxyindole oxidation products of hydrochloric acid eluates were measured by spectrophotofluometry. The levels of norepinephrine and epinephrine were determined by oxidizing the catecholamines at two pH levels and reading the fluorescence at

TABLE I. Urinary and Adrenal Gland Catecholamines After Exposure to 5° for Six Hours.

	Warm (34°)	Cold (5°)
Adrenal catecholamine content (μg/g)	1617 ± 178	1484 ± 102
Adrenal norepinephrine/epinephrine	2.55 ± 0.29	2.03 ± 0.16
Urine catecholamine content (μg/liter)	63 ± 17	160 ± 21[a]
Urine norepinephrine/epinephrine	3.15 ± 1.43	1.84 ± 0.23
Change in rectal temperature (Δ°)	0	−2.1 ± 0.5°[a]
Number of piglets	6	6

[a] $p < 0.05$.

two wavelengths (6).

Tyrosine hydroxylase activity (TH) was assayed radiometrically in 2.5% homogenates of whole adrenal glands prepared in 1.12% KCl–5 m*M* Tris–HCl buffer (pH 6.7) (7). The homogenates were dialyzed overnight at 5° against 5 m*M* Tris–HCl buffer (pH 7.0) containing 1 m*M* mercaptoethanol. The incubation mixture contained: 0.1 ml of 1 *M* Tris–maleate buffer (pH 5.7), 0.1 ml of 5 m*M* $Fe(NH_4)_2(SO_4)_2$, 0.1 ml of 10 m*M* 6,7-dimethyl-5,6,7,8-tetrahydropterine (DM PH_4) dissolved in 1 *M* mercaptoethanol, 0.4 ml tissue homogenate, 0.1 ml of 1 m*M* L-tyrosine containing 10–20 μCi/ml of 3,5-^{3}H-tyrosine, 0.2 ml glass distilled water. The mixture was incubated at 37° for exactly 10 min, the reaction was stopped by adding 0.1 ml of 30% trichloroacetic acid and the precipitated protein was removed by centrifugation. Five-tenths milliliter of the supernate followed by 1.0 of water was placed on a 5 × 30 mm Dowex 50W-X_4 H^+ column. The radioactivity in 0.5 ml of eluate was determined in a liquid scintillation spectrometer using 15 ml of Bray's solution as the counting media. The efficiency of counting was 28%.

Phenethanolamine *N*-methyltransferase (PNMT) was assayed in supernates (40,000*g* for one hour) obtained from the dialyzed homogenates prepared for TH assays (8). The reaction mixture contained the following: 0.1 ml of 0.5 *M* Tris–HCl buffer (pH 8.5), 0.05 ml of 30 m*M* phenethanolamine in 0.01 *N* HCl, 0.05 ml of a solution containing 0.02 μmole *S*-adenosyl methionine and 0.05 μCi *S*-adenosyl methionine-$^{14}CH_3$, 0.1 ml water, 0.2 ml of tissue supernate. The mixture was incubated by adding 0.5 ml of 0.5 *M* sodium borate buffer. The enzyme product was extracted with toluene-isoamyl alcohol (30:1), and an aliquot was assayed for radioactivity in a liquid scintillation spectrometer. The efficiency of counting in a media containing 0.4% Omnifluor (New England Nuclear) in toluene was 88%.

Enzyme assay conditions. The conditions used to assay TH and PNMT in these studies were established with preparations obtained from whole adrenal glands of 35-day-old swine. The rate of TH activity was linear within the range of 0–20 mg of adrenal tissue, while PNMT rates were linear within the range of 0–10 mg of tissue. The activity of PNMT was very low (< 1%) in preparations where no exogenous substrate was added (test for endogenous substrates) or where phenethylamine was used as the substrate (test for nonspecific *N*-methyltransferase activity).

Results. Cold stress. The urine of 3-hour-old fed piglets exposed to 5° for six hours contained more catecholamines than the urine of littermates held at thermoneutrality (34°). The urinary epinephrine/norepinephrine ratios appeared to be the same in "warm" or "cold" animals, although the data from warm animals was quite variable. Adrenal gland catecholamine levels were not altered by the six-hour exposure to cold (Table I).

Figure 1 illustrates the effects of long-term handling and cold exposure upon adrenal-gland catecholamine levels and the activities of PNMT and TH. Piglets, 24 hours old, taken from two different litters were treated in the following ways: 1. unhandled—the animals were left with the sow and not disturbed until they were sacrificed; 2. handled (34°)—the piglets were removed from the sow each morning and afternoon for five con-

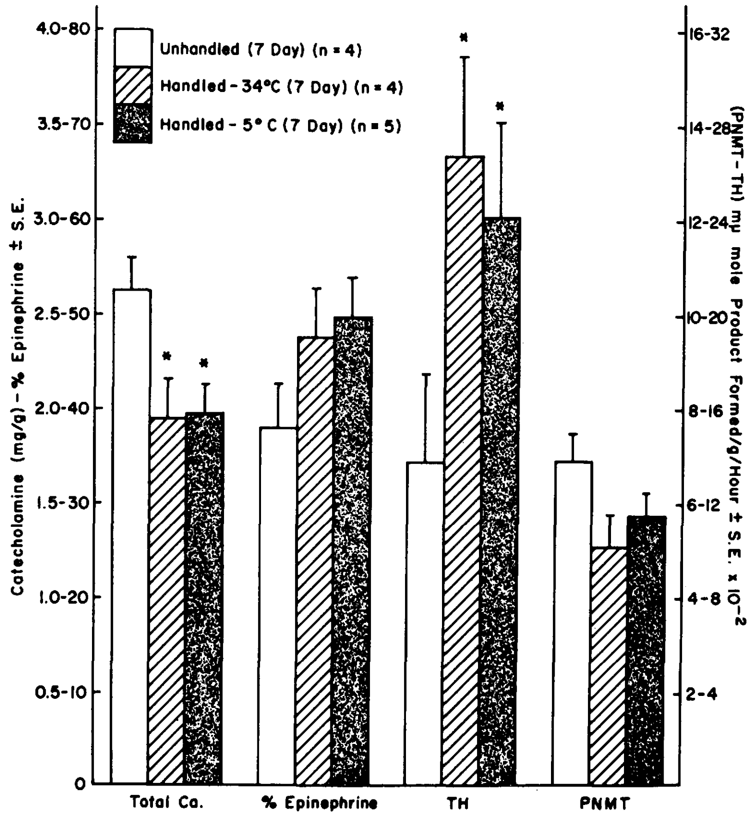

FIG. 1. Effects of repeated (5-day) handling and cold stress on catecholamine levels (Ca), tyrosine hydroxylase activity (TH) and phenethanolamine *N*-methyltransferase activity (PNMT) in the adrenal glands of neonatal swine. An asterisk (*) indicates statistically significant differences ($p < 0.05$) from unhandled controls as determined by an analysis of variance. N = number of animals.

secutive days, transported to the laboratory, maintained at 34° in individual metal containers for 2½ hr and then returned to the sow; 3. handled (5°—piglets treated in the same way as the warm group except they were sequentially placed every morning and afternoon at 34° for ½ hr in individual metal containers in the cold room at 5° for one hour, in individual containers at 34° for one hour and then returned to the dam. The animals in each group were sacrificed when they were seven days of age. Total adrenal-gland catecholamine levels were significantly ($p < 0.05$) lower and TH activities higher in handled than in unhandled animals regardless of whether the piglet had been exposed to 34 or 5°. There were no significant ($p > 0.05$) changes in PNMT activities or in the percent of epinephrine in the adrenal glands.

6-OH Dopamine. Figure 2 illustrates the effects of 6-OH dopamine (50 mg/kg, s.c.) or 0.9% NaCl solution (0.5 ml) administered on two consecutive days to newborn (3-hour-old) piglets. Three of the animals from this litter were left with the sow and not handled. The piglets were sacrificed when they were three days old (24 hr after the last injection). Although the total adrenal gland catecholamine levels were significantly lower ($p < 0.05$) in handled than in unhandled animals, TH activity was only elevated in those piglets which had been treated with 6-OH dopamine. PNMT activity and the

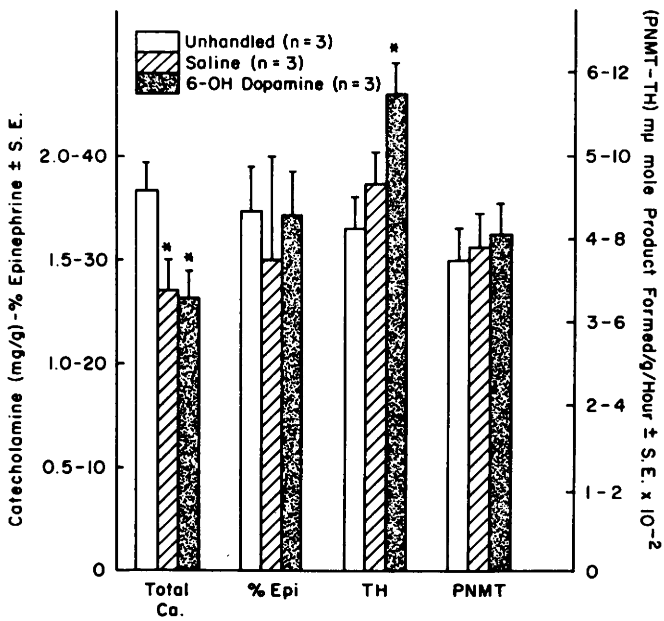

FIG. 2. Effects of 6-hydroxydopamine (50 mg/kg, s.c., daily $\times$ 2 days), 0.9% NaCl solution injections (saline) or no treatment (unhandled) on catecholamine levels (Ca), tyrosine hydroxylase (TH) activity or phenethanolamine *N*-methyltransferase (PNMT) activity in adrenal glands of one litter of newborn piglets. An asterisk (*) indicates a statistically significant difference ($p <$ 0.05) from unhandled controls as determined by an analysis of variance. N = number of animals.

percent of epinephrine in the adrenal glands were the same in all piglets regardless of handling or drug treatment.

Discussion. The temperature needed to induce maximal metabolic changes in the newborn piglet is near 5° (9). Some animals maintain homeothermistry below this temperature but most piglets lose body temperature rapidly when exposed to cold of this severity. (9, 10). The increase in urinary catecholamines observed in the present study in newborn swine exposed to 5° for six hours demonstrates that the sympathetic nervous system was activated, and the high percentage of epinephrine found in the urine implies that a portion of the urinary catecholamines originated from the adrenal medulla. Catecholamine levels in the gland were not detectably altered after six hours exposure to 5°, although the measurement of hormone levels may be quite misleading. Perhaps catecholamine synthesis was increased in the present experiments to match the rate of depletion.

Handling stress may have as much influence on adrenal medullary activity as cold exposure in swine if depletion of adrenal catecholamine levels and enhanced enzyme activity are valid criteria of adrenergic activation. It is not possible to determine, from our experiments, whether repeated cold exposure increased TH activity of the adrenal gland and decreased caecholamine levels since handling may have masked the responses to cold. TH activity (11) and adrenal-gland catecholamine levels (2) have been shown in rats to be significantly altered by relatively short, single exposures to 3–4°, while adrenal-gland TH activity is increased in mice by psychosocial stress (12). The behavior of the newborn piglet suggests that he is quite disturbed by handling. Furthermore, many growing swine die when exposed to the excitement, crowding and exertional stress which they encounter at the time of slaughter (porcine stress syndrome-sudden death syndrome) (13). It is not surprising that TH activity was increased by the handling of the newborn of this species.

TH activity was enhanced in rat adrenal glands following the administration of 6-hydroxydopamine (14). This drug also increased TH activity in the neonatal swine in our studies. Handling (0.9% NaCl solution injections) decreased adrenal catecholamine levels as much as 6-hydroxydopamine injections, although this limited handling did not significantly increase TH activity ($p > 0.05$). However, the tendency toward increased TH activity in handled animals suggests an increase similar to that seen after the more intensive treatment shown in Figure 1.

Adrenal-gland PNMT activity increased in mice of the C57 Bl/Ka strain exposed to 4° for as little as three hours. This seems to be the only species where rapid changes in this enzyme are encountered after stress (15). PNMT activity was not enhanced in the baby piglet even though the animal had been stressed for five days at a level sufficient to cause twofold increases in adrenal gland TH activity.

Summary. Catecholamine content was increased in the urine of newborn swine exposed to 5° for six hours. Adrenal gland hormone levels were not changed under these conditions. Repeated handling (five days) depleted catecholamines and increased the *in vitro* activity of tyrosine hydroxylase in adrenal glands of neonatal piglets. Intermittent exposure to 5° for five days was no more effective than handling in inducing enzyme activity or depleting adrenal gland catecholamines. Tyrosine hydroxylase activity was enhanced in neonatal swine treated with 6-hydroxydopamine for two days. Adrenal catecholamine levels were lower in saline and 6-hydroxydopamine-treated animals than in unhandled controls. Phenethanolamine *N*-methyltransferase activity in the adrenal gland was not altered by handling, cold stress or 6-hydroxydopamine.

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