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Chronic Isoproterenol Treatment of Neonatal Rats

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Abstract ☐ The chronic subcutaneous administration of isoproterenol to neonatal and young male and female rats resulted in statistically significant reductions of the norepinephrine concentrations of the heart and submaxillary and parotid glands and increased concentrations in the stellate ganglion. However, the total norepinephrine content of these tissues was not affected. The epinephrine levels of these tissues were not significantly different from controls except for an increased total content in the submaxillary glands. Chronic treatment did not alter the norepinephrine levels of the brain, superior cervical ganglion, adrenal glands, vas deferens, uterus, spleen, kidneys, lungs, and small intestine. Growth of neonatal rats was retarded. The wet weights of the submaxillary and parotid glands were significantly greater, while the spleen, uterus, adrenal glands, and stellate ganglion weighed less in isoproterenol-treated than in control rats. The wet weights of other peripheral tissues were not affected. The ratios of the tissue weight to the body weight were significantly increased for the heart and submaxillary and parotid glands and decreased for the uterus. The first dose of isoproterenol produced hypothermia in mature rats but hyperthermia in newborn rats. Sparse hair growth in neonatal rats and hair loss in weanling rats were also observed during chronic treatment.

Keyphrases ☐ Isoproterenol, chronic administration—effects on tissue catecholamine levels, tissue weights, and rectal temperature in rats ☐ Catecholamine tissue levels—effect of chronic isoproterenol administration, rats ☐ Tissue weight and catecholamine levels—effect of chronic isoproterenol administration, rats

The ability of isoproterenol to produce hypertrophy and hyperplasia of the submaxillary and parotid glands of rats has been studied by various investigators (1–5). In addition, isoproterenol treatment resulted in infarct-like lesions and fatty degeneration of the heart (6, 7). Effects in other species have also been reported, e.g., cats (8), hamsters (9), guinea pigs (10), and mice (11, 12). Growth stunting, premature opening of the eyes, and an increase of the ratio of heart weight to body weight have been reported following the chronic administration of isoproterenol to neonatal rats (5). Since acute and short-term treatment resulted in increased organ weights and derangements of catechol-

amine levels (13–15) and storage (15), and chronic isoproterenol treatment produced growth stunting and changes in organ weights in neonatal rats (5), the effects of chronic isoproterenol treatment on the tissue catecholamine levels, tissue weights, and rectal temperature were studied in neonatal rats and the results are reported in this paper.

METHODS

Animals—Two days after birth, Sprague–Dawley male and female rats from three litters were subcutaneously dosed with 0.85% sodium chloride solution (controls) or 5 mg./kg. isoproterenol twice a day for 6 days and thereafter with 15 mg./kg. twice a day for up to 43 days. Animals of two other litters received 10 mg./kg. subcutaneously twice a day for 7 days, the first dose being given on the 6th day after birth. At the end of the 1st week of drug administration, nearly 50% of the animals of this group had died and the dose was reduced to 10 mg./kg. once a day.
To obtain additional information on the hyperthermia, the frequently observed lethargy, and the sparsity of hair growth (or hair loss), animals of three other litters were treated chronically by three different dose regimens: 20 mg./kg. once a day and 5 mg./kg. twice a day for 5 days (treatment was started on the 6th day after birth) and 15 mg./kg. twice a day for 30 days (treatment was started on the 25th day after birth).
Catecholamine Analysis—For the tissue catecholamine measurements, the treated animals and their littermate controls were sacrificed by exsanguination under sodium pentobarbital anesthesia after 30–43 days of treatment. The heart, submaxillary glands, parotid glands, spleen, kidneys, lungs, small intestine, vas deferens, uterus, adrenal glands, whole brain (or brainstem[1]), superior cervical ganglia, and stellate ganglia were dissected in the cold and weighed[2]. Tissues were analyzed immediately or frozen and analyzed within 3 days after dissection. Norepinephrine and epinephrine were extracted with 1-butanol as previously described (16) and measured spectrophotofluorometrically by a modification (17, 18) of the ferricyanide oxidation method of von Euler and Floding (19).

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[1] In this report the brainstem is defined as the medulla, the pons, the midbrain, and the diencephalon.
[2] On a Mettler balance or a Cahn electrobalance.

Table I—Wet Weights of Peripheral Tissues and Ratios of Rats Chronically Treated with Isoproterenol[a]

Tissue	Number of Rats	Control Wet Weight, mg. ± SE	Control Ratio, Tissue/Body Weight, mg./g. ± SE	Number of Rats	Isoproterenol Wet Weight, mg. ± SE	Isoproterenol Ratio, Tissue/Body Weight, mg./g. ± SE
Submaxillary glands	17	268 ± 22	3.0 ± 0.1	14	736 ± 138[b]	9.5 ± 0.8[c]
Parotid glands	14	235 ± 32	2.2 ± 0.2	11	843 ± 277[d]	8.6 ± 1.4[c]
Heart	18	404 ± 41	4.1 ± 0.1	14	432 ± 53	6.0 ± 0.2[c]
Spleen[e]	6	398 ± 33	4.7 ± 0.8	4	293 ± 19[d]	4.9 ± 1.0
Uterus	6	142.5 ± 32.7	0.95 ± 0.17	4	53.3 ± 14.3[d]	0.48 ± 0.02[d]
Adrenal[e] glands	8	21.3 ± 1.6	0.24 ± 0.02	6	16.5 ± 0.6[b]	0.25 ± 0.02

[a] Treatment was started 2 and 6 days after birth for 30–43 days. [b] $p < 0.01$, t test in nonpaired experiments (26). [c] $p < 0.001$, t test in nonpaired experiments (26). [d] $p < 0.05$, t test in nonpaired experiments (26). [e] Treated for 30 days.

Table II—Norepinephrine and Epinephrine Concentration and Content of Peripheral Tissues from Control and Chronic Isoproterenol-Treated Rats (Treatment 30–43 days)

Tissue	Treatment	Number of Rats	Micrograms per Gram of Tissue Norepinephrine	Micrograms per Gram of Tissue Epinephrine	Micrograms per Tissue Norepinephrine	Micrograms per Tissue Epinephrine
Heart	Control	18	0.75 ± 0.07[a]	0.15 ± 0.06	0.31 ± 0.06	0.06 ± 0.03
	Isoproterenol	14	0.54 ± 0.04	0.06 ± 0.02	0.24 ± 0.04	0.05 ± 0.02
Submaxillary glands	Control	17	1.00 ± 0.09[b]	0.23 ± 0.07	0.30 ± 0.04	0.04 ± 0.01[c]
	Isoproterenol	14	0.41 ± 0.06	0.13 ± 0.02	0.31 ± 0.06	0.08 ± 0.01
Parotid glands	Control	14	1.03 ± 0.10[b]	0.20 ± 0.02	0.26 ± 0.05	0.04 ± 0.01
	Isoproterenol	11	0.44 ± 0.07	0.18 ± 0.03	0.30 ± 0.10	0.12 ± 0.04

[a] $p < 0.02$, t test in nonpaired experiments (26). [b] $p < 0.001$, t test in nonpaired experiments (26). [c] $p < 0.01$, t test in nonpaired experiments (26).

Table III—Norepinephrine and Epinephrine Concentration and Content of Superior Cervical and Stellate Ganglia of Control and Chronic Isoproterenol-Treated Rats

Ganglia	Treatment	Number of Rats	Weight, mg./Pair	Micrograms per Gram Norepinephrine	Micrograms per Gram Epinephrine	Nanograms per Tissue Norepinephrine	Nanograms per Tissue Epinephrine
Superior cervical	Control	12	1.70 ± 0.12	21.9 ± 2.3	1.3 ± 0.2	35.9 ± 3.0	2.0 ± 0.3
	Isoproterenol	11	1.54 ± 0.12	21.8 ± 2.2	1.4 ± 0.3	33.6 ± 4.8	2.1 ± 0.4
Stellate	Control	12	2.40 ± 0.10[a]	14.9 ± 0.9[a]	0.7 ± 0.2	35.3 ± 1.7	1.7 ± 0.4
	Isoproterenol	11	1.87 ± 0.15	21.1 ± 1.5	0.8 ± 0.3	38.5 ± 3.5	1.4 ± 0.4

[a] $p < 0.01$, t test in nonpaired experiments (26).

Temperature—The rectal temperatures were measured at room temperature (23 ± 1°) with a telethermometer[3] before the isoproterenol administration and every 30 min. for up to 210 min. after dosing. An arterial probe[3] was used for measuring the rectal temperature of newborn rats and thermister probes[4] were used for older animals.

RESULTS

Body Weight—Chronic isoproterenol treatment of neonatal rats for up to 43 days resulted in growth retardation. When treatment was started 6 days after birth with 10 mg./kg., a mean difference in body weight (9%) became apparent after 5–6 days and statistically significant after 7–8 days of treatment (14%; $p < 0.05$). With continuation of the treatment for 15 days, the mean body weight of the isoproterenol-treated group amounted to 69.5% of that of control littermates ($p < 0.01$). When isoproterenol treatment with 5 mg./kg. twice a day was started on the 2nd day after birth and then increased to 15 mg./kg. twice a day, slowing of growth was first observed after approximately 8 days of treatment and a statistically significant difference was observed at 11 days. Growth retardation was still evident at the time of sacrifice and amounted to 15% when compared to control littermates.

Tissue Wet Weights and Ratios—Chronic isoproterenol treatment affected the wet weights and/or the ratios of the tissue weight to the body weight (milligrams per gram) of several organs (Table I). The wet weights of the submaxillary and parotid glands were

increased while the spleen, uterus, and adrenal glands of treated rats were smaller than those of controls. The absolute weights of the hearts were similar in the treated and the control groups, but the ratios of the wet weight to body weight were elevated. These ratios were also increased for the submaxillary and parotid glands, unaltered for the spleen and adrenal glands, and decreased for the uterus.

Catecholamines—Similarity of results on the catecholamine levels from rats treated for 30–43 days permitted the pooling of these data (Table II). Significant decreases in the norepinephrine concentrations of the heart and submaxillary and parotid glands were noted after chronic isoproterenol treatment, but the norepinephrine contents of these tissues were not altered. Statistically significant changes of epinephrine concentrations of the heart, submaxillary glands, and parotid glands did not occur, but the epinephrine content of the submaxillary glands was increased. The catecholamines of other peripheral tissues such as the spleen, uterus, vas deferens, kidneys, lungs, pancreas and adrenal glands, whole brain, and brainstem did not differ from control values. Although the catecholamine concentration and content of superior cervical ganglia were not altered, the norepinephrine concentration of stellate ganglia was increased after chronic treatment (Table III); this was apparently due to a significant reduction in wet weight.

Rectal Temperature—The administration of the first dose of isoproterenol (5 or 20 mg./kg. subcutaneously) to 6-day-old rats resulted in a statistically significant increase in the rectal temperature that lasted about 90 min. Subsequent doses administered once or twice a day for several weeks did not significantly accentuate the hyperthermia (Tables IV and V). Sex difference in the temperature effect of isoproterenol was not studied in detail. However, no statistically significant difference was found in the hyperthermic

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[3] Yellow Springs Instrument Co., Inc.
[4] Nos. 401 and 402, Yellow Springs Instrument Co., Inc.

Table IV—Effect of the Acute and Chronic Isoproterenol Treatment on the Rectal Temperature of Neonatal Rats

Treatment	Age, days	Number of Doses	Number of Rats	Minutes after Isoproterenol Treatment						
				0	30	60	90	120	150	210
				Temperature ± SE						
Controls	6–9	0	12	35.3 ± 0.1°	36.3 ± 0.1°	35.9 ± 0.1°	36.2 ± 0.1°	36.1 ± 0.2°	35.9 ± 0.1°	35.8 ± 0.1°
Isoproterenol, 5 mg./kg.	6	1	6	35.5 ± 0.1°	37.2 ± 0.1°[a]	37.0 ± 0.0°[a]	37.5 ± 0.1°[a]	37.0 ± 0.2°[b]	35.3 ± 0.2°[c]	35.5 ± 0.2°
Isoproterenol, 20 mg./kg.	6	1	4	35.3 ± 0.1°	37.0 ± 0.1°[a]	37.0 ± 0.1°[a]	37.3 ± 0.2°[a]	36.7 ± 0.1°[c]	36.3 ± 0.0°[b]	35.7 ± 0.1°
Isoproterenol, 5 mg./kg.	7	3	6	35.2 ± 0.1°	37.1 ± 0.0°[a]	37.0 ± 0.2°[a]	36.9 ± 0.0°[a]	36.3 ± 0.2°	35.7 ± 0.1°	35.7 ± 0.1°
Isoproterenol, 20 mg./kg.	8	3	4	35.4 ± 0.1°	37.7 ± 0.1°[a]	37.8 ± 0.0°[a]	37.8 ± 0.1°[a]	37.7 ± 0.1°[a]	37.4 ± 0.1°[a]	35.6 ± 0.1°
Isoproterenol, 5 mg./kg.	9	7	6	35.8 ± 0.1°[b]	37.9 ± 0.1°[a]	38.0 ± 0.1°[a]	37.7 ± 0.1°[a]	37.2 ± 0.1°[a]	36.8 ± 0.2°[a]	36.6 ± 0.2°[b]

[a] $p < 0.001$, experimental versus control animals; t test in nonpaired experiments (26). [b] $p < 0.01$, experimental versus control animals; t test in nonpaired experiments (26). [c] $p < 0.02$, experimental versus control animals; t test in nonpaired experiments (26).

Table V—Effect of Acute and Chronic Isoproterenol Treatment on Rectal Temperature of Rats

Treatment	Age, days	Number of Doses	Number of Rats	Minutes after Isoproterenol Treatment					
				0	30	60	90	120	150
				Temperature ± SE					
Control	12–13	—	6	35.1 ± 0.4°	35.0 ± 0.3°	35.3 ± 0.3°	35.7 ± 0.2°	---	—
Isoproterenol, 15 mg./kg.	12–13	12–14	7	34.1 ± 0.4°	36.0 ± 0.7°	36.6 ± 0.6°	37.1 ± 0.1°[a]	---	—
Control	17–18	—	6	36.9 ± 0.1°	36.8 ± 0.1°	36.9 ± 0.2°	36.9 ± 0.2°	--	—
Isoproterenol, 15 mg./kg.	17–18	21–23	7	36.0 ± 0.2°[b]	38.5 ± 0.4°[b]	38.0 ± 0.4°[c]	37.4 ± 0.4°	—	—
Control	24	—	6	37.6 ± 0.2°	37.6 ± 0.2°	37.6 ± 0.2[c]	37.7 ± 0.2°	37.7 ± 0.2°	37.6 ± 0.3°
Isoproterenol[d], 15 mg./kg.	24	35	11	36.1 ± 0.2°[a]	38.4 ± 0.3°[c]	38.4 ± 0.3°[c]	38.1 ± 0.2°	37.5 ± 0.1°	37.1 ± 0.1°
Control	Adult	—	3	37.3 ± 0.4°	37.3 ± 0.4°	37.4 ± 0.2°	37.2 ± 0.3°	37.4 ± 0.6°	37.4 ± 0.3°
Isoproterenol, 15 mg./kg.	Adult	1	3	37.0 ± 0.2°	37.1 ± 0.3°	36.2 ± 0.3°[c]	35.4 ± 0.3°[e]	35.2 ± 0.4°[c]	35.1 ± 0.3°[b]

[a] $p < 0.001$, experimental versus control animals; t test in nonpaired experiments (26). [b] $p < 0.01$, experimental versus control animals; t test in nonpaired experiments (26). [c] $p < 0.05$, experimental versus control animals; t test in nonpaired experiments (26). [d] No statistically significant difference in the hyperthermic response between five male and six female rats. [e] $p < 0.02$, experimental versus control animals; t test in nonpaired experiments (26).

response between young male and female rats after 35 doses (five male and six female rats).

The hyperthermic response following the a.m. administration of the chronic dose was similar to the response of the second daily dose (p.m. administration), which was given about 8 hr. later.

The predose rectal temperature of chronically treated young rats was significantly lower than the comparable rectal temperature of control animals (Table V). In mature rats the first dose of isoproterenol (15 mg./kg.) produced hypothermia (Table V).

Other Observations—In neonatal rats, chronic treatment produced premature opening of the eyelids and scanty fur development. When treatment was started after weaning, marked hair loss was observed usually on the head, back, and/or flanks in approximately half of the treated animals. After chronic dosing, weanling and adult rats became lethargic. The lethargy was interspersed by brief periods of activity which appeared similar to that described by Ingenito and Bonnycastle (20) for dl-4-chloro-N-methylamphetamine. These authors described the activity as "a peculiar backing up and circling movement." Occasionally, the rats in the present study also exhibited a seemingly purposeless repetitious scratching motion of the wire grid with the forepaws.

Of 54 rats from five litters (14 female and 15 male treated rats and 14 female and 11 male control rats) that were dosed for 30–43 days, seven animals died (five female and two male treated rats). No deaths occurred in three other litters which were treated to obtain additional information on the hyperthermia, lethargy, and hair loss.

DISCUSSION

Growth stunting of neonatal rats treated with isoproterenol was reported by Schneyer and Shackleford (5). Despite the large difference in the doses used by these authors and the dose regimens used in the present investigation, the initial growth retardation was similar in both studies. Schneyer and Shackleford (5) administered 1.5 mg./rat twice a day; depending upon the weight of the young rats, this amounted to approximately 30–200 mg./kg. With these large doses, little effect on growth was seen during the first 7 days, but growth was retarded by 15–20% after 15 days.

According to Schneyer and Shackleford (5), animals treated for 35 days were only one-half the size of control littermates. Such profound growth retardation was not encountered with the smaller doses used in the present study.

The enlargement of the submaxillary and parotid glands and the relative increase in the heart weight (in relation to the body weight) noted in the present study were in agreement with the changes reported by Schneyer and Shackleford (5). In mice, however, the wet weights and ratios were increased for the hearts of male but not female mice (21). The smaller wet weights of the spleen and the adrenal glands observed in young chronically treated rats were in proportion to the somatic growth retardation and, therefore, the ratios were not different from those of the controls.

Since isoproterenol is a potent catecholamine closely related to epinephrine in structure and action, an endocrinological effect mediated via the hypothalamic–hypophysial–adrenal or ovarian axis could be responsible for the decreased uterine weight and the smaller tissue to body weight ratio after chronic treatment. A direct action on the uterine smooth muscle cannot be ruled out. In adult mice (21) and rats[s], uterine wet weights showed mean decreases, but these reductions were statistically nonsignificant. However, it is possible that chronic isoproterenol treatment during

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neonatal development may have a more pronounced or even a different effect than in adult animals.

The absence of a decrease in the total norepinephrine content of the heart and submaxillary and parotid glands in the presence of decreased concentrations and increased tissue weights following chronic isoproterenol treatment could be best explained on the basis of the hypertrophy and/or hyperplasia. However, evidence presented by Mueller and Axelrod (15) indicated that short-term isoproterenol treatment resulted in an accelerated release of norepinephrine from the nerve endings of the heart. A storage defect and an increase in sympathetic activity appeared to be responsible for the decrease in cardiac norepinephrine (15). If this postulate can be extended to long-term treatment that is unaccompanied by changes in the total norepinephrine content, an additional, perhaps adaptive, mechanism must be assumed. It should be recalled at this point that only the cardiac norepinephrine concentration and the ratio (tissue weight to body weight) but not the norepinephrine content and the absolute weight were affected by the treatment.

In view of the fact that the norepinephrine content of the stellate ganglion was similar to that in control rats, the increased norepinephrine concentration cannot be readily interpreted without the acquisition of additional data. The smaller wet weight of the stellate ganglia could be explained in terms of differences in the body weights between treated and control littermates; the difference in ganglionic wet weight amounted to 23% and the difference in body weight to 26%. However, the superior cervical ganglion, the wet weight of which is very uniform among littermates, showed only a small statistically nonsignificant decrease after treatment (Table III).

The diversity of the responses of the rectal temperature following acute and chronic isoproterenol treatment in young and adult rats is difficult to interpret. Pretreatment with α- and β-adrenergic receptor blocking agents prevented or ameliorated the hyperthermia in adult rats, but these agents, when administered alone, produced hypothermia (22). The role of epinephrine and related amines on body temperature, control of body temperature, and heat production has been extensively studied and reviewed (23, 24). The direction of the change in body temperature following the administration of amines (as well as other drugs) seems to depend upon the species, route of administration, and dose. Different ambient temperatures before and after drug administration and the predrug body temperature of the experimental animal can also influence the direction of the change. The different response in adult and neonatal rats to isoproterenol observed in this study requires further exploration. Opposite responses in young and adult chickens have been reported with acute doses of epinephrine, norepinephrine, and several other amines (25). Previous investigations almost exclusively dealt with acute drug administration. Little information is available in regard to chronic drug use, body temperature, and temperature regulation.

The mortality rate encountered in the current investigation was similar to rates (5–30%) seen in several studies carried out in this laboratory when neonatal, young, and adult rats were chronically treated with relatively low doses of isoproterenol (5–15 mg./kg.)[6]. No deaths occurred in young female rats (Wistar strain, 110 ± 2.4 g.) treated for 12 days with large doses of isoproterenol: 50 mg./kg. twice a day and 400 mg./kg. once a day (2).

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