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Adrenal Lipid Profiles of Chemically Sympathectomized Normoxic and Hypoxic Neonatal Rats

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Abstract

Neonatal hypoxia is a common condition that elicits a coordinated endocrine response. In the neonatal rat, hypoxia induces an ACTH-independent increase in corticosterone which can be partially blocked by chemical sympathectomy. The present study sought to characterize the effects of sympathectomy on the adrenal lipid profile, since previous work suggested that augmented plasma corticosterone during hypoxia may be due to changes in adrenal lipid metabolism. Newborn rats were exposed to normoxia or hypoxia from birth to seven days of age, and guanethidine was used to produce the sympathectomy. Plasma epinephrine and norepinephrine were not significantly affected by hypoxia, while guanethidine decreased plasma norepinephrine in normoxic and hypoxic pups. Hypoxia alone increased the concentration of cholesterol esters in the adrenal gland; this increase was due to increases in cholesterol ester-associated oleic (18:1n9), docosahexaenoic (22:6n3), arachidonic (20:4n6), and adrenic (22:4n6) acids. Hypoxia also increased diglyceride-associated adrenic acid. Guanethidine treatment attenuated the hypoxia-induced increase in cholesterol ester-bound arachidonic and adrenic acids. Guanethidine also decreased saturated fatty acid concentrations and increased n3 fatty acid-enriched triglyceride. The results support the idea that the ACTH-independent corticosterone response to hypoxia in the neonatal rat is mediated by specific, sympathetically driven alterations in the adrenal lipid profile.

Keywords

fatty acids; steroidogenesis; norepinephrine; epinephrine; adrenocortical

INTRODUCTION

A coordinated endocrine response, along with cardiovascular and respiratory responses, is required for survival in the hypoxic neonate (1–3). Several common neonatal heart, lung, and hemodynamic disorders may cause prolonged hypoxia requiring intensive care (2,4). The hypothalamic-pituitary-adrenal (HPA) axis is a critical component of the neonatal stress response (5–7).

Hypoxia from birth elicits an increase in basal corticosterone concentrations in neonatal rats without an increase in plasma adrenocorticotrophic hormone (ACTH) (8). Chemical

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sympathectomy with guanethidine attenuated this increase in corticosterone (8). We have also shown that hypoxia from birth alters the adrenal lipid profile (9). The purpose of the present study was to characterize the effects of chemical sympathectomy on the adrenal lipid profiles of normoxic and hypoxic neonates. We hypothesized that hypoxia-induced changes in adrenal lipid metabolism would be partially reversed by chemical sympathectomy. Plasma norepinephrine and epinephrine were measured as an index of the chemical sympathectomy, as well as to discern the effects of hypoxia on these hormones.

MATERIALS AND METHODS

In Vivo Experiments

All animal protocols were approved by the Institutional Animal Care and Use Committees of Aurora Health Care and the Medical College of Wisconsin. Timed-pregnant Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN; N=16) were obtained at 14 days gestation and maintained on a standard diet and water ad libitum in a controlled environment (0600–1800 lights on). As soon as a litter was completely delivered, the dam and her pups were immediately moved to an environment chamber and exposed to normobaric normoxia (21% O₂; room air) or hypoxia (12% O₂) as described previously (9). A total of 16 litters (12 pups per litter; mixed sexes) were used.

Pups were treated with guanethidine monosulfate (Sigma Chemical, St. Louis, MO) or vehicle as described previously (8). On the morning of postnatal day (PD) 3, pups were weighed and injected with isotonic saline (vehicle) or guanethidine sc (20 mg/kg). Pups were weighed and injected again on the morning of PD5 (40 mg/kg) and on the afternoon of PD6 (40 mg/kg). Experimentation was performed on the morning of PD7, at which time pups were quickly decapitated and trunk blood collected into lithium heparin. Blood from two pups was pooled for each sample. Adrenal glands were quickly removed, snap frozen in liquid N₂, and pooled (24 adrenals per sample). Animals were divided into four separate groups as follows: normoxia-vehicle (NV), hypoxia-vehicle (HV), normoxia-guanethidine (NG), and hypoxia-guanethidine (HG).

Lipid Profiling

Pooled adrenal glands (~25 mg/sample; 4 independent samples per each of 4 treatment groups) were subjected to lipid profile analyses (Lipomics Technologies, Inc., West Sacramento, CA) using thin layer and gas chromatography as described previously (9).

Hormone Assays

Plasma corticosterone concentrations were measured using a radioimmunoassay (MP Biomedicals, Orangeburg, NJ) as described previously (8). Plasma catecholamine concentrations were measured by a refinement of a tissue method used previously (10,11). Samples for catecholamine analysis were extracted with acid-washed alumina containing 3,4-dihydroxybenzylamine (DHBA) as an internal standard. Standards and quality control pools were extracted and analyzed with every group of samples. Epinephrine and norepinephrine were measured by HPLC and electrochemical detection using an isocratic pump (Hitachi L-7000, San Jose, CA), a Rheodyne 7125 manual injector (Supelco, Bellefonte, PA), a Chromat-integrator (Hitachi D-2500), a Supelcosil LC18 Column (Supelco), and an electrochemical detector (Bioanalytical Systems LC-4B, West Lafayette, IN). The mobile phase was 25 mM citric acid, 25 mM sodium phosphate dibasic anhydrous, 1 mM disodium EDTA, and 40–45 mg/L sodium octyl sulfate at a flow rate of 0.75 mL/min at 25 °C. The electrochemical detector settings were 650 mV applied potential, 1nA sensitivity, and 0.4 offset. Intra-assay variability was 5.5% and 7.9% for norepinephrine and epinephrine, respectively (N=7). Inter-assay variability was 7.4% and 9.3% for norepinephrine and

epinephrine, respectively (N=34). The recoveries of 50 pg standard added to plasma samples were 79% for norepinephrine and 68% for epinephrine. The number of samples assayed for corticosterone and catecholamines were as follows: NV (11), HV (7), NV (12), and HG (11).

Statistics

Quantitative data ($\mu\text{mol/g}$ specimen) from lipid profiles were analyzed with the SurveyorTM software (Lipomics Technologies). Significance of differences between hypoxic and normoxic samples was further evaluated by unpaired Students t-test ($P < 0.05$ considered significant) validated for this type of lipid profile analysis (9,12). Results are reported as mean $\pm$ SEM or as percent change from the corresponding control (heat-map). Hormone data were analyzed by two-way analysis of variance, with *post hoc* analyses performed by Student-Newman-Keuls method for multiple comparisons (SigmaStat 2.03).

RESULTS

Hypoxia did not have a significant effect on plasma norepinephrine concentrations, but significantly increased plasma corticosterone from 11 ± 1 to 30 ± 3 ng/mL. Guanethidine resulted in a similar significant decrease in plasma norepinephrine in normoxic (from 1779 ± 121 to 978 ± 81 pg/mL) and hypoxic pups (from 1654 ± 338 to 974 ± 130 pg/mL). Plasma epinephrine concentrations were not significantly affected by hypoxia or guanethidine ($P > 0.05$); however, there was a tendency for hypoxia to increase epinephrine (from 2521 ± 283 to 3586 ± 1042 pg/mL). Guanethidine had no effect on plasma corticosterone in normoxic pups; guanethidine resulted in lower corticosterone concentrations in hypoxic pups (20 ± 2 ng/mL) when compared to vehicle-treated hypoxic pups.

Figure 1A shows the heat map comparing the effects of hypoxia (HV vs. NV) on the adrenal lipid profile, with the corresponding data listed in Table 1A. First note that all but one of the significant changes due to hypoxia was an increase and that most of these occurred in the cholesterol ester class. Hypoxia significantly increased the concentration of cholesterol ester-bound oleic (18:1n9), docosahexaenoic (22:6n3), arachidonic (20:4n6), and adrenic (22:4n6) acids. The concentration of diglyceride-associated adrenic acid was also increased by hypoxia. Figure 1B provides a summary heat map, highlighting the effects of hypoxia on entire lipid classes or fatty acid families. Hypoxia significantly increased the total concentration of cholesterol esters, and this was due to increased esterification of polyunsaturated fatty acids (of the n3 and n6 families).

Figure 1C shows the effects of guanethidine treatment on the adrenal lipid profile of normoxic pups (NG vs. NV; corresponding data in Table 1B). Note that treatment with guanethidine did not cause any increases in lipid concentrations. Guanethidine decreased the concentration of linoleic acid (18:2n6) in the cardiolipin and lysophosphatidylcholine classes. The concentration of cholesterol ester-bound adrenic acid was significantly decreased by guanethidine treatment.

The heat map in Figure 1D depicts changes in the adrenal lipid profile of hypoxic, guanethidine-treated pups (HG vs. HV). Table 1C lists the corresponding data for this heat map. In the triglyceride class alone, guanethidine increased the concentration of α -linolenic (18:3n3), eicosapentaenoic (20:5n3), docosahexaenoic, and linoleic acid. Guanethidine treatment in hypoxic pups decreased the concentration of palmitic (16:0) and stearic (18:0) acid in the free fatty acid class, and also decreased the concentration of arachidonic and adrenic acid in the cholesterol ester class. Figure 1E shows the summary heat map for the HG vs. HV comparison. Guanethidine treatment in hypoxic pups decreased saturated fatty acids in the cardiolipin, free fatty acid, and phosphatidylethanolamine classes, decreased monounsaturated fatty acids in the lysophosphatidylcholine and phosphatidylethanolamine classes, and decreased polyunsaturated fatty acids in the sphingomyelin class.

DISCUSSION

This study evaluated the effects of chemical sympathectomy on the adrenal lipid profile of normoxic and hypoxic neonatal rats. Hypoxia alone modified the adrenal lipid profile in a manner consistent with the facilitation of steroid production, via specific increases in cholesterol ester concentration/fatty acid composition. The attenuation of some of these changes with guanethidine may explain the effects of sympathectomy on hypoxia-induced corticosterone production.

The present study found no effect of hypoxia on plasma norepinephrine or epinephrine. The tendency towards an increase in epinephrine agrees with the ability of neonatal adrenomedullary chromaffin cells to respond to hypoxia directly (13). Guanethidine treatment decreased plasma norepinephrine in normoxic and hypoxic pups, consistent with this drug's ability to interrupt post-ganglionic, sympathetic neurotransmission (14). Innervation of the epinephrine-secreting adrenal medulla is pre-ganglionic, insensitive to the effects of guanethidine, and not complete until beyond the second week of life in the rat (15). Post-ganglionic innervation of the adrenal cortex and its associated vasculature is present in the neonatal period, is sensitive to the effects of guanethidine, and can modulate steroidogenesis (15). We have shown that neither hypoxia nor chemical sympathectomy had an effect on total adrenal catecholamine content, which primarily represents the adrenal medulla (8).

How could the above findings translate into changes in steroid output? We chose to examine adrenal lipid profiles since we have previously shown that hypoxia has a general effect on intermediary metabolism (5). Hypoxia increased the total concentration of cholesterol esters in the adrenal. Specific increases in major fatty acid metabolite concentrations in the cholesterol ester class included 18:1n9 (oleic acid), 22:6n3 (docosahexaenoic acid), 20:4n6 (arachidonic acid), and 22:4n6 (adrenic acid). Each of these fatty acids has been shown to play roles in membrane permeability, signal transduction, and cellular metabolism (16). Arachidonic acid released from the cleavage of cholesterol esters has been shown to influence mitochondrial cholesterol transport, thereby enhancing steroid production (17). Increased polyunsaturated fatty acids in the diglyceride class of the hypoxic adrenal may act as mediators of signaling pathways involved in intracellular calcium release, which if elevated over time, could lead to sustained stimulation of steroidogenesis (18). Therefore, hypoxia-induced, ACTH-independent increases in corticosterone production may be due to shifts in adrenal lipid metabolism that favor mitochondrial cholesterol transport.

Chemical sympathectomy partially attenuated the corticosterone response to hypoxia, possibly through a decrease in StAR protein expression (8). It is known that hormone-sensitive lipase (HSL) is under the control of catecholamine concentrations (19), and that the adrenal cholesterol esterase enzyme is identical to HSL (20). It is possible that sympathetically driven activation of adrenocortical cholesterol esterase during hypoxia is effectively attenuated by guanethidine. Of most interest was the guanethidine-induced decrease in 20:4n6 concentration in the cholesterol ester class, providing a possible link between sympathectomy and attenuation of steroid production in hypoxic pups (i.e. decreased cholesterol transfer). The degree of saturation of adrenal cardiolipin, free fatty acid, and phosphatidylethanolamine was also decreased following sympathectomy in the hypoxic pup, and these changes may alter membrane dynamics (16), attenuating steroidogenesis via an alternate pathway. Sympathectomy per se in normoxic pups caused a limited number of changes in the lipid profile of the adrenal, and had no effect on plasma corticosterone.

Hypoxia from birth to seven days of age increases basal levels of corticosterone via an ACTH-independent, guanethidine-sensitive mechanism (8). Our results indicate that this increase may be due to altered adrenal lipid metabolism. These alterations, in turn, are at least partly due to

changes in sympathetic discharge. At this time, we cannot delineate with certainty the exact mechanism through which this occurs, nor can we rule out other factors, but we can speculate on possible mechanisms. It is possible that hypoxia maintains increased basal levels of corticosterone through moderate stimulation of the immature adrenal medulla (i.e. a tendency for increased plasma epinephrine). Alternatively, hypoxia may increase direct post-ganglionic input into the developing adrenal cortex. This phenomenon would not affect plasma norepinephrine, but would remain sensitive to the effects of guanethidine. In conjunction, these findings provide a scenario for the primary drive in the maintenance of elevated basal corticosterone levels during hypoxia, which are likely critical to the survival of the developing animal.

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Figure 1. Effects of hypoxia and/or guanethidine on the adrenal lipid profile of neonatal rats
 The concentration of each metabolite from samples of each of the four treatment groups (N=4 per group) was used to generate a heat map. Column headers list different fatty acid metabolites as they occur in the individual lipid classes listed on the left (Panels A, C, and D). Panels B and E show heat maps that summarize changes according to fatty acid families or lipid classes as a whole. The magnitude of the difference in the quantitative data (see Table 1), expressed as percent change from control, is represented by color according to the legend. Differences not meeting $P < 0.05$ are shown in black. Lipid classes in which there were no significant differences were not included in the heat maps ($P > 0.05$). Panels A and B (HV vs. NV), panel C (NG vs. NV), panels D and E (HG vs. HV), SAT=total saturated fatty acids, MUFA=total monounsaturated fatty acids, PUFA=total polyunsaturated fatty acids. Note that guanethidine treatment had no significant effects on any of the summary measurements for the normoxic adrenal ($P > 0.05$; data not shown).

Table 1
Statistically significant, lipid class-specific changes in fatty acid concentrations (nmol/g tissue) in neonatal adrenal glands.

Lipid Class	Fatty Acid	Normoxia-Vehicle	Hypoxia-Vehicle	P
(A)				
CL	22:5n3	199 ± 30	146 ± 22	0.03
	18:1n9	2015 ± 193	2456 ± 279	0.04
CE	20:1n9	432 ± 23	515 ± 48	0.03
	22:6n3	2181 ± 281	2801 ± 215	0.01
DG	18:3n6	33 ± 7	43 ± 5	0.05
	20:4n6	2680 ± 165	3342 ± 323	0.02
	22:4n6	4631 ± 481	6160 ± 496	0.01
	22:4n6	42 ± 13	100 ± 31	0.02
		Normoxia-Vehicle	Normoxia-Guanethidine	
(B)				
CL	18:2n6	1310 ± 196	808 ± 309	0.04
	20:0	289 ± 25	219 ± 34	0.02
CE	22:4n6	4631 ± 481	3646 ± 441	0.02
	15:0	78 ± 31	29 ± 7	0.04
LPC	18:0	1053 ± 76	687 ± 193	0.02
	22:6n3	22 ± 6	12 ± 5	0.03
PE	18:2n6	168 ± 60	71 ± 13	0.04
	18:3n3	13 ± 2	8 ± 1	0.02
(C)				
CL	14:0	319 ± 76	164 ± 68	0.02
	16:0	2732 ± 480	1520 ± 195	0.01
CE	18:1n9	2005 ± 374	1282 ± 183	0.02
	18:3n6	15 ± 4	41 ± 15	0.03
	15:0	321 ± 63	209 ± 36	0.03
	20:3n9	116 ± 7	89 ± 10	0.01
	20:4n6	3342 ± 323	2568 ± 346	0.02
DG	22:4n6	6160 ± 496	4885 ± 469	0.01
	20:4n6	257 ± 31	191 ± 43	0.05

Lipid Class	Fatty Acid	Normoxia-Vehicle	Hypoxia-Vehicle	P
FFA	15:0	50 ± 8	36 ± 4	0.03
	16:0	1936 ± 227	1493 ± 227	0.03
	18:0	1005 ± 174	603 ± 45	0.01
LPC	18:1n9	381 ± 73	167 ± 24	0.01
PC	18:0	7159 ± 885	5540 ± 256	0.02
	24:0	62 ± 29	10 ± 8	0.03
	18:3n3	15 ± 2	10 ± 1	0.01
	18:2n6	2043 ± 114	1862 ± 82	0.05
	20:4n6	8951 ± 1191	7003 ± 432	0.04
PE	22:4n6	309 ± 45	239 ± 20	0.04
	16:0	1623 ± 178	1354 ± 50	0.05
SM	18:0	345 ± 105	178 ± 38	0.04
	18:2n6	71 ± 26	32 ± 14	0.05
TG	14:0	13907 ± 2214	24355 ± 6485	0.04
	18:3n3	2057 ± 443	3347 ± 559	0.01
	20:4n3	167 ± 25	257 ± 49	0.02
	20:5n3	338 ± 101	595 ± 109	0.01
	22:6n3	1438 ± 505	2230 ± 375	0.05
	18:2n6	35206 ± 6443	47578 ± 7505	0.05

Adrenal tissue was subjected to lipid profiling (N=4 per treatment group). CL=cardiolipin, CE=cholesterol ester, DG=diglyceride, FFA=free fatty acid, LPC=lysophosphatidylcholine, PE=phosphatidylethanolamine, PC=phosphatidylcholine, SM=sphingomyelin, TG=triglyceride. Common names of some of the above fatty acids include: 14:0=myristic acid, 16:0=palmitic acid, 18:0=stearic acid, 18:1n9=oleic acid, 18:3n3=alpha-linolenic acid, 20:5n3=eicosapentaenoic acid (EPA), 22:6n3=docosahexaenoic acid (DHA), 18:2n6=linoleic acid, 20:4n6=arachidonic acid, 22:4n6=adrenic acid.