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Differential effects of inhalation exposure to PM_{2.5} on hypothalamic monoamines and corticotrophin releasing hormone in lean and obese rats

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Abstract

Acute exposure to airborne pollutants, especially particulate matter (PM_{2.5}) is known to increase hospital admissions for cardiovascular conditions, increase cardiovascular related mortality and predispose the elderly and obese individuals to cardiovascular conditions. The mechanisms by which PM_{2.5} exposure affects the cardiovascular system is not clear. Since the autonomic system plays an important role in cardiovascular regulation, we hypothesized that PM_{2.5} exposure most likely activates the paraventricular nucleus (PVN) of the hypothalamus to cause an increase in sympathetic nervous system and/or stress axis activity. We also hypothesized that these changes may be sustained in obese rats predisposing them to higher cardiovascular risk. To test this, adult male Brown Norway (BN) rats were subjected to one day or three days of inhalation exposures to filtered air (FA) of concentrated air particulate (CAP) derived from ambient PM_{2.5}. Corpulent JCR-LA rats were exposed to FA or CAP for four days. Animals were sacrificed 24 hours after the last inhalation exposure. Their brains were removed, frozen and sectioned. The PVN and median eminence (ME) were microdissected. PVN was analyzed for norepinephrine (NE), dopamine (DA) and 5-hydroxyindole acetic acid (5-HIAA) levels using HPLC-EC. ME was analyzed for corticotrophin releasing hormone (CRH) levels by ELISA. One day exposure to CAP increased NE levels in the PVN and CRH levels in the ME of BN rats. Repeated exposures to CAP did not affect NE levels in the PVN of BN rats, but increased NE levels in JCR/LA rats. A similar pattern was observed with 5-HIAA levels. DA levels on the other hand, were unaffected in both BN and JCR/LA strains. These data suggest that repeated exposures to PM_{2.5} continue to stimulate the PVN in obese animals but not lean rats.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Keywords

Particulate matter; repeated exposure; sympathetic nervous system; stress axis; cardiovascular risk

1. Introduction

Exposure to particulate matter (PM) is known to increase the risk for cardiovascular diseases worldwide (1). Exposure to fine PM such as PM_{2.5} affects heart rate variability in experimental rodents (15, 32), the elderly (7, 21) and also in young healthy males (24) suggesting that it affects cardiac autonomic control. The mechanism by which PM_{2.5} exposure affects cardiovascular is unclear. There has been some evidence to suggest that inflammatory changes in the lungs that results in the release of chemical mediators could contribute to the changes in autonomic nervous system (ANS) control of cardiac rhythm (13). Besides affecting heart rate variability, PM_{2.5} exposure is also known to increase blood pressure in normotensive healthy adults (41) and especially in patients with preexisting cardiovascular disease (CVD) (42). These observations suggest that the association between PM_{2.5} exposure and CVD risk may be due to decreased vagal or increased sympathetic tone (24).

One of the important central sites for sympathetic nervous system (SNS) regulation is the paraventricular nucleus (PVN) of the hypothalamus. The PVN has efferent connections to the intermediolateral cell column of the thoracic and lumbar spinal segments that in turn connects to various parts of the body through pre and post ganglionic neurons(8). The SNS is responsible for increasing heart rate and blood pressure during stressful situations or in response to external stimuli and is an essential part of the homeostatic mechanism (2). Activation of the PVN could therefore be a mechanism by which PM exposure increases SNS activity to affect cardiovascular functions.

Besides playing an important role in SNS regulation, the PVN is also a key player in regulating stress axis activity. The PVN receives rich noradrenergic, dopaminergic and serotonergic innervations from the hindbrain. These neurotransmitters are important for activation of corticotrophin releasing hormone (CRH) neurons (27) that are located in the PVN and CRH is released from terminals in the median eminence (ME). We have previously observed that acute exposure to PM_{2.5} can increase NE levels in the PVN which could have implications in both sympathetic nervous system (SNS) and stress axis activity (39). However, it is not clear if PM_{2.5} will continue to increase NE levels with repeated exposures of 3 days or more. Since the stress axis is capable of adapting to stressful stimuli (29), we hypothesized that repeated exposure to PM_{2.5} would result in subdued changes in NE levels in the PVN. We wanted to verify downstream changes in stress axis activity by using CRH levels in the ME. Besides producing serious health effects in the elderly, harmful effects of PM_{2.5} exposure are more pronounced in obese individuals (9). This could be due to elevated stress axis or SNS activity. To study this, we used spontaneously obese JCR/LA-cp rats that are corpulent, have hyperlipidemia and are insulin resistant (34). We hypothesized that PM exposure would produce marked increases in NE levels in the PVN of these rats. To test these hypotheses, we exposed Brown Norway (BN) rats to 1 and 3-day exposures of PM_{2.5} and exposed JCR/LA rats for 4 days to PM_{2.5}. We measured NE and other monoamines in the PVN and CRH levels in the ME.

2. Materials and Methods

2.1 Animals and Treatment

Adult male BN rats were obtained from Charles River Laboratories (Portage, MI). Four month old JCR/LA rats were obtained from Charles River laboratories as well. A group of 8 month old JCR/LA rats were kindly donated by Dr. J.C. Russell, University of Alberta. Animals were exposed to PM_{2.5} as described earlier (17, 39) using a mobile laboratory (AirCARE1) (10) located either at Calvin College in Grand Rapids, Michigan (BN rats), or at Maybury Elementary School in Detroit, Michigan (JCR rats). The inhalation lab contains a Harvard/U.S. Environmental Protection Agency ambient fine particle concentrator and 2 stainless steel Hinners-type whole body chambers capable of holding 16 rats. The chambers could hold a volume of 0.32 m³ of air. PM_{2.5} was generated using the EPA concentrator with air drawn from the local urban atmosphere. One of the chambers was used for PM_{2.5} exposure, while the other was used for exposing animals to HEPA-filtered clean air at the same flow rate (control) (17). In the first experiment, BN rats were exposed to CAP for 1 or 3 days in Grand Rapids, MI and sacrificed 24hrs after exposure by pentobarbital administration. JCR/LA rats were exposed to PM_{2.5} for 4 days in Detroit in another experiment. At the time of sacrifice, body weight was measured, blood was collected and the brains were removed, frozen and sectioned as described before (39). All the protocols used in this experiment were approved by the Institutional Animal Care and Use Committee at Michigan State University.

2.2 Characteristics of PM 2.5

Continuous ambient PM_{2.5} concentration data were collected using a tapered Element Oscillating Microbalance (TEOM) monitor (Rupprecht and Patashnick, Model 1400AB). Major components of organic and elemental carbon, sulfates, nitrates, ammonia and crustal/urban dust were determined as previously described (17).

2.3 Brain microdissection

Serial sections (300µm thickness) of the brains were obtained using a cryostat (Slee Mainz, London, UK) on clear glass slides. The sections were then placed on a cold stage (−10°C) and the hypothalamic nuclei of interest, namely the PVN and ME, were microdissected using the Palkovit's microdissection technique with a 500µm diameter punch. We used the Rat Brain Stereotaxic Atlas as a reference (30). The co-ordinates for microdissecting the PVN and ME were −1.8 to −2.12 mm and −2.12 to −3.3 mm posterior to the bregma respectively.

2.3 Neurotransmitter analysis using HPLC

NE and other monoamines in the PVN were measured using a Shimadzu Prominence UFLC system (Shimadzu, Columbia, MD). Briefly, it consisted of a LC-20 AD Prominence Pump, a DGu-20A3 degasser, a SIL 20AC autosampler, and a CTO 20AC column oven maintained at 37°C. We used a ODS reverse phase C-18 column (Phenomenex, Torrance, CA) for the separation of neurotransmitters. The mobile phase contained 14.5 g of chloroacetic acid, 0.3 g of octane sulfonic acid, 0.25 g of ultrapure EDTA, 4.675 g of sodium hydroxide, 17.5 ml of acetonitrile and 14 ml tetrahydrofuran per litre with pH adjusted to 3.1. The flow rate of the mobile phase was set at 1.8 ml/minute. The PVN punches were homogenized in 60µl 0.1M perchloric acid and 5µl of the homogenate was used for protein estimation using bicinchoninic acid assay (Pierce, Rockford, IL) as described earlier. The remaining homogenate was centrifuged at 13000rpm for 10 minutes and 15µl of the supernatant was injected into the HPLC system using an autoinjector. 15µl of Dihydroxybenzylamine (0.05M) was added to the supernatants as an internal standard. The chromatograms were

analyzed using the Class VP software version 7.4 SP3 (Shimadzu, Columbia, MD). NE and other monoamine values were expressed as pg/ μ g of protein.

2.4 ELISA

CRH levels in the ME were measured using a competitive ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA). Samples were assayed in duplicates as per the manufacturer's instructions. CRH levels were expressed as pg/ μ g of protein.

2.5 Statistical analysis

Differences in body weight between BN and JCR rats were compared using ANOVA followed by Fisher's LSD post hoc analysis. Differences in neurotransmitter levels and CRH levels between different exposures in BN rats were compared by ANOVA followed by Fisher's LSD test. Neurotransmitter values in 4 month old and 8 month old JCR/LA rats were pooled to get a n=8 per group because there were no significant differences between them. Differences in neurotransmitter concentrations and CRH levels in JCR/LA rats were then compared by Student's t test.

3. Results

3.1 Body weight

Fig. 1 shows the effects of PM_{2.5} exposure on body weight in BN and JCR/LA rats. Both acute and chronic PM_{2.5} exposure did not affect body weights in BN rats. A similar effect was observed in JCR/LA rats exposed to 4 days of CAPs. JCR/LA rats were significantly heavier than BN rats (Fig 1A) and 8 month old JCR/LA rats were heavier than the 4 month old rats (Fig 1B).

3.2 Comparisons of CAP characteristics between Grand Rapids and Detroit

Table 1 describes differences in PM_{2.5} mass and major components in CAPs collected in Detroit and Grand Rapids. Average mass concentrations of concentrated PM_{2.5} (μ g/m³) in Detroit (291) were almost half of what was generated during exposures in Grand Rapids (519 and 595 for 1 and 3 day exposures respectively). Major components in Detroit PM_{2.5} were fairly consistent throughout the exposure period with little day-to-day variation in organic carbon (OC) and sulfates. By comparison, the last day of repeated exposures in Grand Rapids was marked by high sulfates and total mass. Also, organic carbon in PM_{2.5} was consistently higher in Grand Rapids compared to Detroit, suggesting more input from traffic sources.

3.3 NE levels in the PVN

In BN rats, acute exposure to PM_{2.5} produced a moderate but significant increase in NE levels (mean $\pm$ SE, pg/ μ g protein) in the PVN (13.5 $\pm$ 2.14) when compared to rats exposed to air (8.9 $\pm$ 1.1, p <0.05). While NE levels after repeated exposure to air and PM_{2.5} did not differ from each other (14.3 $\pm$ 0.5 and 14.4 $\pm$ 0.7 in air and PM_{2.5} treated groups respectively), they were significantly different from the group acutely exposed to air. In JCR/LA rats, repeated exposure to PM_{2.5} exposure produced a significant increase in NE levels in the PVN (15.3 $\pm$ 1.6) compared to animals exposed to air (8.7 $\pm$ 0.8, p <0.05) (fig 2A and B).

3.4 Other neurotransmitters in PVN

PM_{2.5} exposure did not affect DA levels in BN and JCR/LA rats (Table 2). In contrast, 5-HIAA levels (Mean $\pm$ SE; pg/ μ g protein) increased 2–3 fold with both acute (7.6 $\pm$ 2.5) and repeated (7.4 $\pm$ 0.2) PM_{2.5} exposure compared to air exposure (2.6 $\pm$ 0.4) in BN rats. A similar

increase in 5-HIAA levels was observed in JCR/LA rats exposed to PM_{2.5} (12±1.6) when compared to animals exposed to air (7.5±1.1) (p<0.01).

3.5 CRH levels in the ME

While acute PM_{2.5} exposure produced a 3-fold increase in CRH levels in BN rats compared to air treated rats, CRH levels after repeated exposure to PM_{2.5} exposure was not significantly different compared to air treated rats. CRH levels after both acute and repeated PM_{2.5} exposure were comparable and were significantly different only from acute air exposed group in BN rats (fig 3A). In JCR/LA rats, PM_{2.5} exposure produced a marked increase in CRH levels compared to animals exposed to air (fig 3B).

4. Discussion

Results from this study indicate that exposure to PM_{2.5} produces a significant increase in NE and 5-HIAA levels in both BN and JCR/LA rats. However, this increase was apparent only after acute exposure in BN rats and was also noted after repeated exposure in JCR/LA rats suggesting that JCR/LA rats have sustained increases in neurotransmitter levels in the PVN. This may translate into chronic increases in sympathetic tone in JCR/LA but not BN rats. In BN rats, stress axis activation was observed after acute exposure as measured by increase in CRH levels in the ME. This response was lost after repeated exposure in these rats. There was a similar pattern in JCR/LA rats after repeated exposure. Taken together, these data suggest that there is an adaptive response in the stress axis after repetitive PM_{2.5} exposures. However, this adaptation does not extend to noradrenergic activity in the PVN in JCR/LA rats suggesting that obesity may contribute to prolonged activation of the SNS after repeated CAPs exposure in these obese animals.

The PVN receives rich noradrenergic innervations from A1 and A2 brainstem NE neurons (36). It has efferent connections to the brainstem and the intermediolateral cell column (IML) of the spinal cord (40) and has reciprocal connections with brain areas that are involved in cardiovascular functions (37, 40). Increases in NE levels in the PVN contribute to sympathoexcitation in rats (43). A number of studies have shown that exposure to PM_{2.5} is strongly associated with cardiovascular mortality (1) and is thought to promote atherosclerosis (18) and cause vascular stiffening (19) leading to increase in blood pressure. Small increases in heart rate and endothelial dysfunction after PM exposure are believed to reflect PM-induced autonomic imbalance (4). PM-induced effects on autonomic functions are reflected as changes in heart rate variability (7), arrhythmias (22), premature ventricular contractions (14) and blood pressure (41). The increases in blood pressure were apparent within a short period of few hours (41). These observations suggest that the SNS may be involved in this phenomenon.

Since the PVN is capable of activating the SNS in response to environmental and physiological influences, it was logical to measure NE levels in the PVN both after single and repetitive exposures to PM_{2.5} in BN rats. The increase in NE levels that were observed after acute exposure agrees with our previously published study (39). The interesting observation in the present study was that this difference between PM_{2.5} exposure and the air-treated group was not apparent after chronic exposure. Since NE levels in this group was comparable to those observed after acute PM_{2.5} exposure, and did not increase further compared to the control group, it is possible that there is an adaptation response to PM_{2.5} exposure. The stress axis is capable of adapting to repetitive stressful episodes (26). Therefore, it is likely that the lack of further increases in NE levels after multiple exposures to PM_{2.5} is part of an adaptive response. In contrast to BN rats, there was almost a 2-fold increase in NE levels after PM_{2.5} exposure compared to air exposure in JCR/LA rats. The reason for increase in NE levels after PM_{2.5} exposure is not clear. It is very likely that PM

exposure can incite an inflammatory process leading to an oxidative stress response within the lungs (3). The resultant release of intermediary molecules such as free radicals (11) and reactive oxygen species can affect central sites specifically the rostroventrolateral medulla (RVLM) to influence blood pressure and cardiovascular functions, since scavenging of superoxide in the RVLM can prevent increase in blood pressure in response to peripheral chemoreflex stimulation with potassium cyanide (28).

CRH is also known to play an important role in cardiovascular and autonomic responses (5). CRH induced cardiovascular excitation is thought to be mediated through sympathetic and vagal efferents (12). Although most CRH neurons located in the PVN are involved in ACTH secretion and stress axis activity (12), some CRH neurons project to the brainstem especially the A1 region or RVLM (25) that is important for cardiovascular regulation. Some CRH neurons also project to the intermediolateral cell column of the spinal cord (35) and these CRH neurons are considered to be preautonomic. Under physiological conditions, very few CRH type 1 receptors that mediate SNS activity are detectable. However they undergo rapid up regulation in the event of stress or increased CRH secretion (23, 31). In BN rats, CRH levels increased after acute exposure to PM_{2.5}. In contrast, CRH levels after repeated PM_{2.5} exposure were not significantly different from the air exposed group. In JCR/LA rats, we observed a similar trend in CRH levels after repetitive PM_{2.5} exposure.

This could be an adaptive response to PM_{2.5} exposure as well. This can also be linked to the obese phenotype of these animals. We have previously observed that NE levels do increase in the PVN without producing any change in CRH levels in the ME of obese rats when they are placed on a high fat diet for long periods of time (38). The lack of a CRH response in the face of elevated NE levels has been attributed to a reduction in α_2 adrenergic receptor levels in the PVN in obese rats (20). A similar phenomenon may be in operation in JCR/LA rats after multiple, repetitive exposure to PM_{2.5} and needs further investigation. The increase in NE levels in obese animals after chronic PM_{2.5} exposures could also be due to the persistent increase in serum lipids in these animals. Elevations in free fatty acids can stimulate NE release in the hypothalamus (our unpublished observation). Since JCR/LA rats have a hyperlipidemic background, the combination of PM_{2.5} exposure-induced stress with higher serum lipid levels could be a cause for the persistence in NE elevations in these animals. The lack of increase in CRH but elevated NE levels in the PVN could favor an increase in SNS activity in these animals predisposing these rats to unexpected cardiovascular outcomes. This difference in responsiveness to PM_{2.5} exposure could place obese individuals at risk for autonomic dysregulation.

Besides affecting SNS activity, the PVN is also the central site for integrating stress responses. Emotional, immunological, and physical stress result in an increase in glucocorticoid secretion. This is brought about by a cascade of events starting with neurotransmitter stimulation of CRH neurons in the PVN, that releases CRH from the ME. CRH enters the portal circulation to stimulate adrenocorticotropin (ACTH) secretion from the pituitary. ACTH acts directly on the adrenal gland to stimulate glucocorticoid secretion (27). Since CRH levels were suppressed after repeated exposures to PM_{2.5} in both BN and JCR/LA rats, it is possible to conclude, that the animals generate an adaptive response to the stress of PM_{2.5} exposure.

The differences in stress axis responses could also be due to variations in the composition of PM_{2.5} between the two sites. Comparison of PM_{2.5} from Detroit and Grand Rapids showed modest differences in both total mass and major constituents. While both exposure sites are located in urban Midwestern cities near major roadways, higher organic carbon in PM_{2.5} from Grand Rapids suggests that this site had greater impact from traffic sources on days of exposure. Furthermore one of the days during exposures in Grand Rapids was marked by a

high sulfate content of ambient PM_{2.5}. How these qualitative differences in CAPs might affect the responses in brain centers that we observed is open to speculation. Since our previous report in BN rats (39), there has been little new information with regard to stress-axis activation in response to airborne particulate matter. Using similar systems, we and others have reported PM_{2.5}-related changes in heart rate variability that is related to metal content (6, 16, 33). We did not analyze metals in the present study and as such their contribution to the stress responses we describe are unknown. It is plausible that, in addition to underlying metabolic differences in JCR versus BN rats, exaggerated stress responses in PM_{2.5} are related in part to specific components. Further studies are needed to confirm the neuroendocrine responses to various components of PM_{2.5}, and elucidate the underlying processes of this adaptive stress response.

Highlights

This manuscript compares the effects of PM_{2.5} exposure on neurotransmitter and corticotrophin releasing hormone (CRH) levels in a lean and obese animal model. We found that

1. There is marked activation of the paraventricular nucleus after acute exposure to PM_{2.5} as indicated by increases in the levels of neurotransmitters such as norepinephrine and 5-hydroxyindole acetic acid. However, this activation is not apparent after 3 day exposure to PM_{2.5} in a lean rat model (Brown Norway rat).
2. CRH levels follow the neurotransmitter pattern in these lean rats as well. Acute exposure to PM_{2.5} produces an increase in CRH levels, while subchronic exposures do not.
3. In obese rats, subchronic exposure to PM_{2.5} increases norepinephrine levels in the paraventricular nucleus but does not increase CRH levels.
4. Possible reasons for these differences between lean and obese rats are discussed.

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Fig 1. Effect of PM 2.5 exposure on body weights in BN and JCR/LA rats

A. Body weights in BN rats compared to JCR/LA rats. There were no treatment differences, but only strain differences. **B.** Body weight differences between the 2 age groups of JCR/LA rats. Older rats were heavier than the younger rats. There were no treatment differences. 'a' indicates $p < 0.05$ compared to BN rats or younger JCR/LA rats.

A **B**

Fig 2. Effect of PM_{2.5} exposure on Norepinephrine levels in the PVN

A. NE levels in BN rats after single and multiple day PM_{2.5} exposure. There were significant increases after 1 day exposure between treatments, but not after 3 day exposure. 'a' indicates significant difference from acute-air treated group, $p < 0.05$. **B.** NE levels in the PVN of JCR/LA rats. There were significant differences between the treatment and control group, 'a' indicates $p < 0.05$.

Fig 3. Effect of multiple day PM2.5 exposure on CRH levels in the ME

A. CRH levels in BN rats after 1 and 3 day PM2.5 exposure. There were significant changes in CRH levels only after PM2.5 exposure for 1 day. 'a' indicates significant difference from acute-air exposed group; $p < 0.05$. No differences were observed in CRH levels after repeated exposures. **B.** CRH levels in the ME of JCR/LA rats. There were no treatment effects on CRH levels in these rats.

Table 1
Comparison of various components of CAPs in the different exposures in Grand Rapids and in Detroit

OC represents organic carbon and EC represents elemental carbon.

Day	Mass	OC	EC	Nitrate	Sulfate	Ammonium	Crustal/Urban dust
BN rats 1 day exposure- Grand Rapids							
Day 1	519	280	7	23	58	51	36
BN rats 3 day exposure- Grand Rapids							
Day 1	388	140	3	4	106	50	14
Day 2	492	222	3	13	59	30	15
Day 3	904	198	8	35	417	81	20
Avg	595	187	4	18	194	54	16
JCR rats 4 day exposure- Detroit							
Day 1	289	128	7	20	59	28	29
Day 2	448	150	16	31	63	20	62
Day 3	251	121	7	36	64	31	35
Day 4	176	92	12	6	21	9	27
Avg	291	123	10	23	52	22	38

Table 2
Neurotransmitter levels in the PVN of PM2.5 exposed BN and JCR/LA rats

Neurotransmitter levels in the PVN were measured by HPLC-EC after 1 day and 3 or 4 day PM_{2.5} exposure in BN and JCR rats. There were no significant changes in DA levels, but 5-HIAA, a metabolite of serotonin increased significantly after repeated PM_{2.5} exposures in both BN and JCR/LA rats.

BN rats	Neurotransmitters	1 day exposure		3 day exposure	
		Air	PM _{2.5}	Air	PM _{2.5}
	Dopamine (pg/µg)	1.8±1.2	2.0±0.7	0.6±0.1	0.6±0.1
	5-HIAA (pg/µg)	2.6±0.4	7.6±2.5*	6.8±0.9*	7.4±0.2*

JCR/LA rats	Neurotransmitters	4 day exposure	
		Air	PM _{2.5}
	Dopamine (pg/µg)	0.5±0.1	0.7±0.1
	5-HIAA (pg/µg)	7.5±1.1	12±1.6*