Effects of Benzo[a]pyrene on Autonomic Nervous System of Coke Oven Workers

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Abstract: Effects of Benzo[a]pyrene on Autonomic Nervous System of Coke Oven Workers: Hong-Mei ZHANG, et al. Department of Occupational Health, School of Public Health, Shanxi Medical University, China—Objectives are to investigate the effects of benzo[a]pyrene (BaP) on the autonomic nervous system of coke oven workers. One hundred eighty-four coke oven workers were divided into 3 groups according to their working sites (coke oven bottom group, coke oven side group and coke oven top group), and 93 referents were recruited. BaP monitored by air sampling pumps as well as urinary 1-hydroxypyrene (1-OH-Py) was determined by high performance liquid chromatograph with a fluorescence detector (HPLC-FD). The autonomic nervous system (ANS) function was determined by 4 tests: Valsalva Maneuuvre heart rate variation (HR-V), variation of heart rate when breathing deeply (HR-DB), variation of heart rate when instantly standing up (HR-IS, including RR30:15 and RRmax:min) and variation of blood pressure when instantly standing up (BP-IS). The BaP mean concentrations in coke oven bottom, coke oven side and coke oven top were 19, 185 and 1,623 ng/m3, respectively. The levels of urinary 1-OH-Py were markedly higher in the 3 exposed groups than that in the referent group (p<0.01). No significant difference was found in each group between smokers and non-smokers (p>0.05). Compared with referents, HR-V decreased significantly in coke oven workers (p<0.01), representing modulation of parasympathetic nervous function. However, no statistical differences were found in HR-DB, RR30:15, RRmax:min and BP-IS between the exposed groups and the control group (p>0.05). HR-V decreased with the increment of 1-OH-Py (p<0.05), and results of multiple linear stepwise regression demonstrated that external exposure level and duration of education entered the HR-V model; age was a significant factor of HR-DB and RRmax:min, but no variable was involved in RR30:15 and BP-IS regression. Benzo[a]pyrene affects the autonomic nervous function of coke oven workers mainly by down-regulating the parasympathetic nervous function. (J Occup Health 2008; 50: 308–316)

Key words: Benzo[a]pyrene, 1-hydroxypyrene, Autonomic nervous system

Benzo[a]pyrene (BaP), a widespread environmental and industrial pollutant, is the most important family member of polycyclic aromatic hydrocarbons (PAHs), and is generally considered a representative marker for studying the adverse effects induced by PAHs. BaP and other PAHs are by-products from incomplete combustion, pyrolysis, and pyrosynthesis of all kinds of organic matters. Industries emitting BaP include production of coke, asphalt and tar, and aluminum electrolysis. Occupational exposure to BaP is mainly through inhalation of particulates in the ambient air of operation areas.

As a classic carcinogen, BaP could remarkably raise the morbidity of lung carcinoma, skin cancer and hepatoma in the occupationally exposed population. However, perhaps too much attention was paid to its carcinogenicity in the past to the extent that other potential toxicities of BaP might have been neglected, such as its neurotoxicity which is gradually being disclosed. BaP can reach the brain tissues by crossing the blood-brain barrier or directly by passing through the olfactory nerve, and is accumulated in the cerebellum. Although
the content of B[a]P in brain is very much less than that in liver or kidney, it is very hard for the organism to eliminate it\(^4\). In view of this fact, we suppose that long-term exposure to B[a]P would harm the nervous system because the nervous system is quite vulnerable to all kinds of toxicants, even at relatively low doses. Studies indicated that morphologic changes, DNA lesion, even cell apoptosis occurred in the nerve tissues resulting in nervous system damage when mice were treated with B[a]P for 8 wk\(^5,6\). The findings of Saunders et al.\(^3\) demonstrated that B[a]P induced acute neurobehavioral toxicity in F344 rats including neuromuscular, autonomic, sensorimotor and physiological symptoms within 2 and 4 h post B[a]P administration. Furthermore, a significant correlation existed between neurotoxic effects and levels of B[a]P in plasma, and concentrations of B[a]P metabolites in the rats’ brain tissues\(^8\). One report said that PAH mixtures, comprising B[a]P, chrysene, anthracene, and pentachlorophenol, were capable of inducing damage in neural cells\(^9\). Gestational exposure to B[a]P aerosol decreased the learning capability in F1 generation rats\(^10\). In a short, all of these reports support the notion that B[a]P may be neurotoxic. Nevertheless, it is extremely difficult to perform advanced research on neurotoxicity of B[a]P. Why? Because on the one hand, there is too little basic research about its neurotoxicity in the human population, due to a lack of necessary occupational epidemiological data; and on the other hand, there are few objective methods to judge the neurotoxicity of PAHs among the occupationally exposed population. Therefore, we decided to apply an autonomic nervous system (ANS) function test battery of relatively simple and sensitive methods, to investigate the neurotoxicity of B[a]P among coke oven workers. The conclusion that the autonomic nerve system is damaged by B[a]P occupational exposure is reported in this paper. Hopefully, this investigation will provide some fundamental data and evidence for sophisticated researches and protecting the health of workers.

**Subjects and Methods**

**Subjects**

All the study participants were employed in a large iron and steel company in Taiyuan, China. Exposed groups were selected from workers who had currently been working for at least 1 yr in the coke plant of the company. The coke oven workers were categorized into 3 external exposure subgroups according to their working sites: coke oven bottom group (low exposure group), coke oven side group (medium exposure group), and coke oven top group (high exposure group). According to the work program, each worker worked consistently in the same site for 6 months, then was rotated to one of the other three working sites. Referents were selected from the warehouse workers working in the raw material division of the same company, 4 km away from the coke plant, who had not visited the coke operation area in last 3 months. Socioeconomic status and general physical condition were comparable between the groups. Also residences of both the exposed groups and the referents were in the same living area provided by the company, which is about 2.5 km away from the coke plant and not located down-wind of it. Written informed consent was obtained from these workers, and the research protocol was approved by the Ethics and Human Subject Committees of Shanxi Medical University.

**Methods**

1) **Questionnaire survey**

A questionnaire (designed by ourselves) was administered to all subjects by a professional investigator face through face-to-face interview to gather general information involving age, educational level, smoking and drinking habits, personal vocational history, individual and family medical histories, medication in the last 2 wk, and the distance between residence and the coke plant. Smoking was defined as currently smoking no less than 10 cigarettes per day for at least one consecutive year; drinking was defined as currently drinking wine, beer or spirits no less than 3 times per week at least for 6 consecutive months. If any question was not replied to clearly or properly in the questionnaire, the subject was asked again.

The study subjects were determined on the basis of information obtained from the questionnaire. The selection criteria were composed of items described below

1) Exposed groups were constantly exposed to B[a]P in their occupational activities. 2) Referents were never occupationally exposed to B[a]P. 3) All subjects were generally not exposed to other occupational hazards at levels which can result in dysfunction of ANS, including noise, vibration, lead and mercury etc. 4) All subjects were in good health during the period of investigation, and had not suffered from diseases and disorders causing ANS dysfunction, including epilepsy, syndrome of neuroasthenia, cerebropathia, heart disease, diabetes, hypertension, hyperthyreosis and so on. 5) Every subject had not taken medicine which would interfere with ANS, such as sympathomimetic or parasympathetic agents and sympathetic or parasympathetic inhibitors in the last 2 wk. Forty-three workers were excluded from the 310 participants and the final study population consisted of 37 coke oven bottom workers, 67 coke oven side workers, 70 coke oven top workers and 93 referents.

2) **Exposure assessment**

Three representative monitoring points were selected in coke oven bottom, coke oven side, coke oven top and one in the referents’ working place in order to measure concentrations of airborne B[a]P. Two air sampling
pumps (HSF513, Germany) were located at each monitoring point and at the height of personal breathing zone to collect parallel air samples. A working shift lasts 8 h and the worker’s task is relatively constant during the shift. The air samples were gathered at the flow rate of 2 l/min for 6 h during the complete working day and for 3 consecutive days in March 2004. The air pressure and temperature were recorded to calculate the standard sampling volume. Then the filters taken away from the sampling pumps were sealed in a clean container and stored at 4°C, then transported to the laboratory. B[a]P in the filters was analyzed with high performance liquid chromatography (LC-10A, SHIMADZU, Japan) after being extracted by hexamethylene 4 times and alkaline chromatography (LC-10A, SHIMADZU, Japan) after being stored at –80°C until analysis. B[a]P in the filters was analyzed with high performance liquid chromatograph (LC-10A, SHIMADZU, Japan) after being extracted by hexamethylene 4 times and alkaline aluminum oxide. The conditions of detection were:

Chromatographic column: hypersil ODS C18 (4.6 mm × 150 mm i. d., 5 µm);
Moving phase: methanol/water=85/15, flow rate: 1.0 ml/min;
Column temperature: 25°C;
Wavelengths for the fluorescence detector: λ ex/λ em = 365/405 nm;
Identification of measured materials was determined by the retention time and quantification was conducted by comparing the peak areas of sample with a standard substance.

3) 1-OH-Py in urine

Post-shift urine samples were collected in 50 ml polyethylene plastic tubes, then stored at –80°C until analysis. 1-OH-Py in urine was determined by a high performance liquid chromatograph (LC-10A, SHIMADZU, Japan) equipped with a fluorescence detector (RF-10AXL, SHIMADZU, Japan). The measurement conditions were as follows:

Chromatographic column: hypersil ODS C18 (4.6 mm × 250 mm i. d., 5 µm);
Moving phase: methanol/water=75/25, flow rate: 1.0 ml/min;
Column temperature: 35°C;
Wavelengths for the fluorescence detector: λ ex/λ em = 346/386 nm;
Identification and quantification of the measured materials were accomplished by comparing retention times and peak area ratios of samples with those of standards. Carbazole was used as an internal standard substance. The detailed analytical method has been presented elsewhere. Identification and quantification of the measured materials were accomplished by comparing retention times and peak area ratios of samples with those of standards. Carbazole was used as an internal standard substance. The detailed analytical method has been presented elsewhere [15]. The lowest limit of detection of urinary 1-OH-Py was 1.0 ng/ml urine. Measurements below a concentration of 1.0 ng/ml were given a value of 1.0 ng/ml ratio to \( \sqrt{2} \).

The urinary creatinine reacted with alkaline picrate, and the creatinine-picrate complex was measured by spectrophotometry (TU-1800S, China) at a wavelength of 520 nm. The concentration of 1-OH-Py was normalized to creatinine and presented as µmol/mol creatinine (Cr).

4) Test of ANS function

Autonomic nervous system (ANS) function was tested in the morning by two physicians in a comfortable and quiet room at a relatively constant temperature (18–20°C) in the Center of Disease Control of the company. The test methods recommended by Ewing [13] included the following 4 items: Valsalva Manoeuvre heart rate variation (HR-V), variation of heart rate when breathing deeply (HR-DB), variation of heart rate after instantly standing up (HR-IS, including RR30:15 and RRmax:min) and variation of blood pressure after instantly standing up (BP-IS). The detailed procedures are described below (1) HR-V: After lying on a bed quietly for 5 min, a subject blew into a manometer, to make it reach 40 mm mercury column, and held it for 15 s, then relaxed and returned to breathing normally. Electrocardiography was recorded continuously. Afterwards, we obtained HR-V by computing R-Rmax:R-Rmin after measuring the maximum R-R distance (R-Rmax) when relaxing and the minimum (R-Rmin) when blowing. (2) HR-DB: A subject was told to sit quietly for 5 min, then breathe deeply at the frequency of 6 times per minute (inhaling for 5 s, exhaling for 5 s), and then to quickly revert to normal respiration. We successively traced the electrocardiogram over the whole process. Similarly, we measured the fastest and the slowest heart rates, then calculated the difference in each respiratory cycle. HR-DB was computed as the average of difference in 6 cycles. (3) HR-IS: As above, electrocardiography was recorded from the beginning to the end. A subject was ordered to stand up quickly after lying on a bed quietly and relaxing for 5 min. First we measured the 30th and the 15th R-R intervals, then we determined RR30:15; Besides RRmax:min was calculated to the ratio value between the maximum R-R span and the minimum one within 30 s after the subject stood up. (4) BP-IS: the procedure resembled that used for HR-IS. A subject’s systolic blood pressure was examined when he was lying on a bed and relaxing and after standing up instantly. The BP-IS value was the difference between those two blood pressures. But if a subject’s electrocardiography was abnormal in the first 5 min, he was excluded from our study.

5) Data input and statistical methods

All data were input into a personal computer with software Epidata. Concentrations of post shift urinary 1-OH-Py were log10 transformed to normalize their distributions before analysis. All statistical methods were carried out with SPSS 10.0 FOR WINDOWS, and all p values were two-sided.
Results

General information of the study subjects (see Table 1)
As shown in Table 1, exposure groups and referents were balanced in age, habits of smoking and drinking (p>0.05), but not in year of education (p<0.01) by one way analysis of variance (ANOVA). Therefore, statistical analysis of covariates should be used in the comparison of ANS between exposure groups and referents.

Results of exposure assessment (see Table 2)
Table 2 shows that concentrations of B[a]P in coke oven side and coke oven top significantly exceeded the limit value, but they were not higher in coke oven bottom or the referents’ workplace. Concentrations of B[a]P rose rapidly from coke oven bottom to coke oven side, and to coke oven top. The reference limit was 0.15 µg/m³ of B[a]P in the workplace air, the maximum allowable concentration in a hygiene standard of the ex-Soviet Union.

Levels of urinary 1-OH-Py and effect of smoking on 1-OH-Py (see Tables 3 and 4)
The results in Table 3 were obtained by one-way ANOVA analysis. They demonstrate that concentrations of urinary 1-OH-Py increased with external exposure levels, and rose significantly in coke oven workers compared to the control group (p<0.01). Also the concentrations were higher in coke oven side and coke oven top workers than in coke oven bottom workers (p<0.05).

Considering that there was a large proportion of smokers in the control group and exposed groups, we analyzed the effect of smoking on the concentrations of 1-OH-Py after dividing the groups into smokers and non-smokers. No significant differences were found in 1-OH-Py levels between smokers and non-smokers in each groups (p>0.05; Table 4).

Changes of ANS function (see Tables 5 and 6)
The results of covariate analysis demonstrated that HR-V decreased sharply in exposed groups compared referents to (p<0.01) after adjusting for year of education, but no significant differences were found in HR-DB, RR30:15, RRmax:min and BP-IS between coke oven workers and referents (p>0.05).

To further analyze the relationship between B[a]P and ANS function, we divided the groups into 0~2.90, 2.90~3.77 and 3.77~5.44 µmol/mol Cr group according to the 25th and the 75th percentiles of 1-OH-Py concentrations. Table 6 shows that HR-V significantly decreased with the 1-OH-Py levels (p<0.05), however, other indices did not show noticeable changes (p>0.05).

Results of multiple linear regressions (see Table 7)
The results of multiple linear regression analysis indicated that log10 transformation of 1-hydroxypyrene concentrations, year of coking and year of education were related to HR-V; age was related to HR-DB and RRmax:min but no any variable was related to RR30:15 or BP-IS. Smoking and drinking were not related in any outcome measure and therefore did not independently affect ANS function. The detailed coefficients are given in Table 7.
Table 3. Levels of urinary 1-OH-Py of study subjects (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Levels of 1-OH-Py (unit: µmol/mol Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referent group</td>
<td>93</td>
<td>2.76 ± 1.02</td>
</tr>
<tr>
<td>Coke oven bottom group</td>
<td>37</td>
<td>3.21 ± 0.77a</td>
</tr>
<tr>
<td>Coke oven side group</td>
<td>67</td>
<td>3.57 ± 0.58a,b</td>
</tr>
<tr>
<td>Coke oven top group</td>
<td>70</td>
<td>3.66 ± 0.61a,b</td>
</tr>
</tbody>
</table>

*: denotes that levels of urinary 1-OH-Py in coke oven bottom group, coke oven side group and coke oven top group were significantly higher than that of referents (p<0.01); b: denotes that, levels of urinary 1-OH-Py in coke oven side group and coke oven top group were significantly higher than that in coke bottom group (p<0.05).

Table 4. Effects of smoking on the concentrations of 1-OH-Py (µmol/mol Cr) (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Smokers</th>
<th>Non-smokers</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referents</td>
<td>29</td>
<td>64</td>
<td>0.517</td>
<td>0.606</td>
</tr>
<tr>
<td>Coke oven bottom group</td>
<td>15</td>
<td>22</td>
<td>0.750</td>
<td>0.458</td>
</tr>
<tr>
<td>Coke oven side group</td>
<td>15</td>
<td>52</td>
<td>–0.706</td>
<td>0.482</td>
</tr>
<tr>
<td>Coke oven top group</td>
<td>30</td>
<td>40</td>
<td>–0.501</td>
<td>0.618</td>
</tr>
<tr>
<td>Total groups</td>
<td>89</td>
<td>178</td>
<td>0.773</td>
<td>0.440</td>
</tr>
</tbody>
</table>

Table 5. Changes of ANS function among coke oven workers (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>HR-V</th>
<th>HR-DB</th>
<th>RR30:15</th>
<th>RRmax:min</th>
<th>BP-IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referents</td>
<td>93</td>
<td>2.41 ± 0.16</td>
<td>9.70 ± 0.60</td>
<td>1.05 ± 0.02</td>
<td>1.31 ± 0.03</td>
<td>2.63 ± 1.96</td>
</tr>
<tr>
<td>Coke oven bottom group</td>
<td>37</td>
<td>1.32 ± 0.25*</td>
<td>10.44 ± 0.92</td>
<td>1.04 ± 0.03</td>
<td>1.30 ± 0.04</td>
<td>4.59 ± 3.02</td>
</tr>
<tr>
<td>Coke oven side group</td>
<td>67</td>
<td>1.32 ± 0.19*</td>
<td>10.47 ± 0.69</td>
<td>1.05 ± 0.02</td>
<td>1.33 ± 0.03</td>
<td>6.53 ± 2.27</td>
</tr>
<tr>
<td>Coke oven top group</td>
<td>70</td>
<td>1.30 ± 0.18*</td>
<td>9.74 ± 0.67</td>
<td>1.04 ± 0.02</td>
<td>1.34 ± 0.03</td>
<td>2.19 ± 2.19</td>
</tr>
<tr>
<td>F value</td>
<td>–</td>
<td>9.627</td>
<td>0.354</td>
<td>0.023</td>
<td>0.652</td>
<td>0.790</td>
</tr>
<tr>
<td>p value</td>
<td>–</td>
<td>0.000</td>
<td>0.786</td>
<td>0.995</td>
<td>0.582</td>
<td>0.500</td>
</tr>
</tbody>
</table>

*: denotes that compared to the referents, significant differences existed (p<0.01).

Table 6. Changes of ANS by stratification of 1-OH-Py levels (µmol/mol Cr) (x ± s)

<table>
<thead>
<tr>
<th>1-OH-Py levels</th>
<th>n</th>
<th>HR-V</th>
<th>HR-DB</th>
<th>RR30:15</th>
<th>RRmax:min</th>
<th>BP-IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2.90 group</td>
<td>69</td>
<td>2.18 ± 2.15</td>
<td>9.59 ± 5.25</td>
<td>1.06 ± 0.13</td>
<td>1.31 ± 0.26</td>
<td>6.28 ± 12.34</td>
</tr>
<tr>
<td>2.90–3.77 group</td>
<td>132</td>
<td>1.56 ± 1.39*</td>
<td>10.25 ± 5.71</td>
<td>1.05 ± 0.18</td>
<td>1.32 ± 0.26</td>
<td>8.21 ± 12.97</td>
</tr>
<tr>
<td>3.77–5.44 group</td>
<td>66</td>
<td>1.45 ± 1.27*</td>
<td>9.94 ± 5.63</td>
<td>1.02 ± 0.14</td>
<td>1.34 ± 0.26</td>
<td>9.17 ± 11.29</td>
</tr>
<tr>
<td>F value</td>
<td>–</td>
<td>4.445</td>
<td>0.322</td>
<td>0.737</td>
<td>0.175</td>
<td>0.974</td>
</tr>
<tr>
<td>p value</td>
<td>–</td>
<td>0.013</td>
<td>0.725</td>
<td>0.480</td>
<td>0.840</td>
<td>0.379</td>
</tr>
</tbody>
</table>

*: represents that compared to the referents, significant differences existed (p<0.05).
sudden death. In fact, Igor Burstyn et al. reported that a cardiac insufficiency, malignant arrhythmia and even coronary heart disease such as myocardial ischemia, largely fasten heart beat, and then cause all kinds of nerve to increase. The heightened sympathetic tone would sympathetic one, and force the activity of sympathetic break the balance between parasympathetic nerve and parasympathetic function would decrease the vagal tone, limited and further cut down. Lower modulation of concentration of B[a]P in that coke plant must be strictly autonomic nervous function of coke oven workers. The neurotoxicants than the sympathetic one17, 19).

parasympathetic nervous system is more vulnerable to agreement with other studies arguing that the showing down-regulation of parasympathetic tone, is in nervous system, was not notably changed. This result, but BP-IS, representing modulation of the sympathetic nervous system of coke oven workers. We found that HR-V, HR-DB, RR30:15 and RRmax:min respond to variation of heart rate and can reflect modulation of the parasympathetic nervous system. BP-IS represents only fluctuation of blood pressure and can indicate regulation of the sympathetic nervous system. The higher these parameters are, the better the autonomic nervous system function is, and vice versa, the lower they are, the worse it is. Nowadays, uses of the ANS test battery is widespread and it has proved to be a useful tool for assessing neurotoxicity of occupational hazards, including metals and organic solvents, among the working population. In this paper we investigated the changes of autonomic nervous system of coke oven workers. We found that HR-V, reflecting regulation of the parasympathetic nervous system, was significantly decreased in exposed groups compared with referents; but BP-IS, representing modulation of the sympathetic nervous system, was not notably changed. This result, showing down-regulation of parasympathetic tone, is in agreement with other studies arguing that the parasympathetic nervous system is more vulnerable to neurotoxicants than the sympathetic one17, 19).

The present study indicates that B[a]P damaged the autonomic nervous function of coke oven workers. The concentration of B[a]P in that coke plant must be strictly limited and further cut down. Lower modulation of parasympathetic function would decrease the vagal tone, break the balance between parasympathetic nerve and sympathetic one, and force the activity of sympathetic nerve to increase. The heightened sympathetic tone would largely fasten heart beat, and then cause all kinds of coronary heart disease such as myocardial ischemia, cardiac insufficiency, malignant arrhythmia and even sudden death. In fact, Igor Burstyn et al. reported that a

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>HR-V</th>
<th>HR-DB</th>
<th>RRmax:min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log10 transformed 1-OH-Py</td>
<td>0.109</td>
<td>-0.149</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>-</td>
<td>-0.138</td>
<td>-0.155</td>
</tr>
<tr>
<td>Year of coking</td>
<td>-0.031</td>
<td>-0.182</td>
<td>-</td>
</tr>
<tr>
<td>Year of education</td>
<td>0.128</td>
<td>0.207</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion

The ANS test battery recommended by Ewing13) is regarded as a sensitive and effective method of detecting neurotoxicity of matters existing in workplaces for a long period14–18). It is easy to perform, highly sensitive, low in cost, and non-invasive. Dysfunction of the autonomic nervous system is a frequent finding in many neurological and internal diseases. Usually, the function of autonomic nervous system was markedly lowered once the nervous system has been damaged. HR-V, HR-DB, RR30:15 and RRmax:min respond to variation of heart rate and can reflect modulation of the parasympathetic nervous system. BP-IS represents only fluctuation of blood pressure and can indicate regulation of the sympathetic nervous system. The higher these parameters are, the better the autonomic nervous system function is, and vice versa, the lower they are, the worse it is. Nowadays, uses of the ANS test battery is widespread and it has proved to be a useful tool for assessing neurotoxicity of occupational hazards, including metals and organic solvents, among the working population. In this paper we investigated the changes of autonomic nervous system of coke oven workers. We found that HR-V, reflecting regulation of the parasympathetic nervous system, was significantly decreased in exposed groups compared with referents; but BP-IS, representing modulation of the sympathetic nervous system, was not notably changed. This result, showing down-regulation of parasympathetic tone, is in agreement with other studies arguing that the parasympathetic nervous system is more vulnerable to neurotoxicants than the sympathetic one17, 19).

The present study indicates that B[a]P damaged the autonomic nervous function of coke oven workers. The concentration of B[a]P in that coke plant must be strictly limited and further cut down. Lower modulation of parasympathetic function would decrease the vagal tone, break the balance between parasympathetic nerve and sympathetic one, and force the activity of sympathetic nerve to increase. The heightened sympathetic tone would largely fasten heart beat, and then cause all kinds of coronary heart disease such as myocardial ischemia, cardiac insufficiency, malignant arrhythmia and even sudden death. In fact, Igor Burstyn et al. reported that a significant positive correlation existed between the accumulative index and average exposure index of workers occupationally exposed to B[a]P and morbidity of fatal ischemic heart disease20). Moreover, the relative risk of fatal ischemic heart disease was 1.64 when the average concentration of B[a]P was 273 ng/m³ or above20). A similar result was confirmed in a population exposed to coal tar. The morbidity and mortality of cardiovascular diseases markedly rose when people exercised in an environment with high concentration of automobile exhausts containing B[a]P20). Therefore, the toxicity of B[a]P on the autonomic nervous system should be paid close attention because it could cause heart disease.

In this study population, smoking and drinking were comparable between groups. Further analysis showed that smoking had no effect on the level of 1-OH-Py although there were 68.82% smokers in the control group. This may be due to coke oven workers inhaling much more B[a]P in the working environment than from smoking, thus hiding the effect of smoking on 1-OH-Py concentration. A study analyzed the effect of smoking on 1-OH-Py concentrations by dividing coke oven workers into three subgroups according to urinary cotinine concentrations (non-smokers, light smokers, and heavy smokers with <0.05, 0.05~0.9, and >0.9 nmol cotinine/mol Cr), and concluded that smoking had no effect on the level of 1-OH-Py concentrations. A study analyzed the effect of smoking on 1-OH-Py concentrations by dividing coke oven workers into three subgroups according to urinary cotinine concentrations (non-smokers, light smokers, and heavy smokers with <0.05, 0.05~0.9, and >0.9 nmol cotinine/mol Cr), and concluded that smoking had no significant effect on 1-OH-Py concentrations of coke oven workers21).

In the present study, the relationship between 1-OH-Py and ANS was further confirmed by dividing the groups according to the 25th and the 75th percentiles of 1-OH-Py concentrations. The result that HR-V decreased with the 1-OH-Py indicates that changes of ANS were related to B[a]P (1-OH-Py). Moreover, the results of multiple linear stepwise regressions indicated that log10 transformed 1-hydroxypyrene, years of coking, years of education and age could influence the changes of heart rate in different directions. Log10 transformed 1-hydroxypyrene and years of coking were both risk factors of ANS. 1-Hydroxypyrene was a metabolite of and reflects the dose of B[a]P exposure, and years of coking could represent the duration of B[a]P exposure. Our results show that dysfunction of the autonomic nervous system of coke oven workers depended on the dose and the duration of B[a]P exposure, although age was an
unavoidable physical agent\textsuperscript{22}, and years of education was a protective factor. So, quality of education, health instruction and protective measures all had positive effects on coke oven workers’ health. To our surprise, the standard partial regression coefficient of log10 transformation of urinary 1-OH-Py was not high and regarding this we make the following observations. First, there were many affecting factors we could not control in the investigation, something which is different from animal experiments. Second, concentrations of post-shift urinary 1-OH-Py were relevant not only to that of pre-shift urine, but were also affected by genetic polymorphisms of many metabolic enzymes including AhR, GSTs and CYP1A1 etc\textsuperscript{23–28}. Third, the level of 1-OH-Py was also affected by individual alcohol consumption\textsuperscript{21, 29, 30}. It was previously reported that coke oven workers who consumed >50 g of ethanol/day had significantly higher 1-OH-Py levels than those who consumed <50 g of ethanol/day\textsuperscript{21}. Another study found that heavy drinkers (>120 g/wk) had lower concentrations of urinary 1-OH-Py than never and former drinkers in the total population\textsuperscript{21}. We deduce from this that alcohol changes toxicokinetic metabolism pathways or transforms 1-OH-Py into other metabolites. The stimulating effect of ethanol on microsomal mono-oxygenases through induction of the CYP2E1 form has been confirmed by animal experiments\textsuperscript{22}. Finally, the half life of urinary 1-OH-Py is from 6 to 35 h. Accordingly, it cannot exactly represent the true concentration of B[a]P after long-term exposure. More importantly, 1-OH-Py is almost exclusively a metabolite of pyrene\textsuperscript{33}, a constituent of PAH exposure. More importantly, 1-OH-Py is almost exclusively a metabolite of pyrene\textsuperscript{33}, a constituent of PAH exposure. However, there was still no better biomarker than 1-OH-Py, so 1-OH-Py still acts as an internal dosage marker of occupational B[a]P exposure. There are few epidemiological documents on neurotoxicity of B[a]P. Neurological symptoms including vegetative dysregulation and loss of short-term memory have been reported in workers following acute and chronic exposure to B[a]P in coke-producing plants in Poland\textsuperscript{34}. Neurological abnormalities were reported in a community exposed to B[a]P, benzofluoranthene, chrysene, naphthol and pyrene and other PAHs dumped at national priority hazardous waste sites in Texas from the 1960s until the late 1970s\textsuperscript{35}. Neurophysiologic and neuropsychological impairments which occurred in residents in Louisiana were related to higher levels of PAHs detected in nearby soils and waters\textsuperscript{36}. Our study revealed that down-regulation of parasympathetic tone among coke oven workers was related to B[a]P in coke operations, but to determine the exact relationship of cause and effect, it is essential to commit to further cohort studies and animal experiments in future.

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