

Original Article

Toxic Chemical from Plastics Attenuates Phenylbiguanide-induced Cardio-respiratory Reflexes in Anaesthetized Rats

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Abstract

Bisphenol A (BPA) attenuated phenylbiguanide (PBG)-induced cardio-respiratory reflexes involving decreased vagal afferent activity. BPA leaches out from plastics thus it is expected that chronic exposure to plastic boiled (PBW) water will also produce similar changes. Therefore, the present study was undertaken to evaluate the effects of chronic ingestion of PBW on PBG evoked reflexes and were compared with BPA. Adult female rats were ingested BPA containing pellets (2 µg/kg body weight)/PBW/tap water (*ad libitum*) for 30 days. On day 30, the animals were anaesthetized and BP, ECG and respiratory excursions were recorded. Further, PBG was injected intravenously to evoke cardio-respiratory reflexes and at the end lungs were excised for histopathological examination. BPA concentration in PBW was 6.6 µg/ml estimated by HPLC. In rats receiving tap water, PBG produced bradycardia, hypotension and tachypnoea. In PBW/ BPA treated groups, PBG-induced reflexes were attenuated significantly along with emphysematous and consolidative changes in lungs. The present results indicate that PBW attenuates the protective cardio-respiratory reflexes and also produces histopathological changes in lungs.

Key words: Alveolar pathology; Emphysematous changes; J-Reflex; PBG-reflex; Plastic boiled water; BPA-toxicity

Introduction

Bisphenol A (BPA) is a chemical used in manufacturing polycarbonate plastics. Beverage

bottles, coating on the inner side of food packaging cans, milk packets, microwave oven wares, etc are made of plastics. BPA leaches out in the aqueous medium at high temperature and acidic pH (1-3). Exposure to BPA occurs mainly through the ingestion of food and water from containers and dental materials made up of plastics that leach BPA. BPA is shown to cross the blood-placental barrier and is found in the maternal blood, amniotic fluid, placental tissue and in umbilical cord blood (4). BPA has been reported to produce toxicity in various biological systems (5-9). In our earlier studies we have shown that BPA decreased contractility of spontaneously

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beating rat atria *in vitro* (10). Also, BPA produced lethality due to respiratory arrest and hypotension (11). Recently, BPA is reported to attenuate the phenylbiguanide (PBG)-induced reflex response by decreasing the vagal afferent activity (12). It is not clear that the decreased activity is due to the histological changes in lungs. Further, since BPA leaches out from plastics, we postulated that exposure of rats to plastic boiled water (PBW) may also produce effects similar to BPA. Hence, the present study was undertaken to determine the effect of PBW on PBG-evoked reflexes and then compared it with BPA. In addition, histopathological examination of lungs was performed in order to determine the structural changes produced by PBW/BPA. Further, the estimation of BPA in PBW was also done by HPLC.

Materials and methods

Animals

Adult female Albino rats of Charles Foster strain weighing 150-200 g were used. The animals were housed in a temperature, humidity (50% of RH) and light controlled room (12:12 h light: dark) and *ad libitum* supply of food (Raj Food Corporation, Varanasi) and water were provided. The animal experiments were performed as per the guidelines given by the Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Experimental protocol

In the present study, the animals were divided into three groups ($n = 6$ in each group). In the first group, the animals were given tap water *ad libitum* (served as control group) for 30 days, whereas the rats of second group received plastic boiled water (PBW) *ad libitum*, for 30 days. The animals of third group received food pellets containing BPA (2 µg/kg/day) for 30 days as reported earlier (12). After 30 days, the experiments were conducted on these animals to elicit PBG-induced cardio-respiratory reflexes.

Dissection and recording of cardio-respiratory reflexes

The trachea, jugular vein and femoral artery were cannulated in urethane anesthetized rats as described earlier (12-14). The tracheal cannulation was performed to maintain the patency of respiratory tract; jugular venous cannulation was done for injecting BPA/PBG and femoral artery cannulation was performed for recording blood pressure via pressure transducer. The respiratory movements were recorded by a force transducer. The electrocardiographic potentials were recorded by needle electrodes (standard limb lead 2 configuration) connected to Bio amplifier. All the recordings were made on computerized chart recorder (AD Instruments, Australia).

After the dissection, the animals were allowed to stabilize for 30 min. Then the initial recordings of blood pressure, ECG and respiration were obtained. Subsequently, PBG (10 µg/kg body weight) was injected via jugular vein and the responses (ECG, respiration and blood pressure) were recorded for 60 s.

At the end of the experimentation, lungs were excised for histopathological examination. Briefly, the lungs were fixed in formal saline, processed for sectioning and mounting. Thin sections were made and stained with hematoxylin and eosin.

Estimation of BPA in PBW

HPLC was performed with two high pressure gradient pumps having a maximum pressure limit of 400 kgf, equipped with a UV-Vis detector (SPD-20a/AV), a 20 µl injection loop and a C₁₈ column (Shimadzu equipment, Japan). The system was controlled and the data were acquired and processed using the LC solution software.

The mobile phase consisted of 50 mM sodium acetate buffer adjusted to pH 4.8 with acetic acid and acetonitrile (1:1 v/v) (15, 16). The flow rate through the column was adjusted to 1 ml/min. Stock solution of standard of BPA (0.1 mg/ml) was made in acetonitrile. Working standard was prepared by

dilution of stock solution in 30% acetonitrile (v/v). Intra-assay precision was calculated as the percentage relative standard deviation (RSD) from the three replicate analyses of the standard in series.

Drugs and solutions

Bisphenol A was obtained from HiMedia laboratory, Pvt. Ltd (Mumbai, India). PBG and urethane were procured from Sigma Aldrich, St. Louis MO; USA. Acetonitrile was obtained from Fischer solutions, Dalles TX, USA. Food pellets containing BPA were prepared by dissolving BPA in vegetable oil then mixed with wheat flour and water as described earlier (12). PBW was obtained by boiling plastic bags (250 g obtained from local vendors) in 500 ml tap water for 60 min. The final volume of the boiled water was made to 500 ml. Animals of PBW group received this water without any dilution.

Data analysis and statistics

The heart rate (HR) and respiratory frequency (RF) were computed manually; mean arterial pressure (MAP) was computed by the software Lab chart 7 (AD Instruments, Australia). HPLC data was computed with calibration for a known concentration of BPA for area and timings of standard BPA peak. The area of PBW peak at that time interval was computed. All the data were pooled and the mean \pm SEM was calculated. The data were compared by two-way ANOVA followed by Student-Newman-Keuls test for multiple comparisons ($P < 0.05$ was considered significant). Student's t-test for paired or unpaired observations was also used as required ($P < 0.05$ was considered significant).

Results

Effect of chronic exposure to BPA and PBW on cardio-respiratory parameters

The heart rate, respiratory frequency and MAP in various subgroups of chronically treated animals after stabilization (prior to PBG injection) are given in Table I. The heart rate and respiratory rate in PBW and BPA groups were not different from the control group.

TABLE I: Mean \pm SEM values of heart rate (HR), respiratory frequency (RF) and MAP in various groups. The control group received tap water for 30 days; BPA treated group received BPA (2 μ g/kg body weight/day; orally) for 30 days; PBW group received plastic boiled water (ad libitum) for 30 days (n = 6 in each group).

	Control	BPA	PBW
HR (beats/min)	340 \pm 12.4	356 \pm 18.3	351 \pm 10.2
RF (breaths/min)	96 \pm 8.5	99 \pm 18.3	91 \pm 11.8
MAP (mm Hg)	103.1 \pm 6.6	108.8 \pm 3.0	83.8 \pm 4.7*

* $P < 0.05$ as compared to control group; * $P < 0.05$ as compared to BPA group (Student's t test for unpaired observations).

However, MAP in PBW group was significantly lesser than control and BPA group ($P < 0.05$; Student's t-test for unpaired observations).

PBG reflex response in control group

Intravenous injection of PBG produced bradycardia, hypotension and tachypnoea in animals receiving tap water (control) group. The PBG-induced changes in heart rate showed an immediate fall by 61% which recovered slowly and at 60 s the fall was 27% (Fig. 1) In case of MAP response, hypotension was maximum (50%) at 15 s which recovered gradually and the decrease was 15% at 60 s (Fig. 1). In case of respiration, there was an immediate rise by about 10% followed by a fall of 20 % (10 15 s) and then rise of 40-50% (Fig. 1).

PBG reflex response in BPA treated group

In BPA ingested group, the time response of PBG-induced reflex changes were attenuated significantly (Fig. 1; $P < 0.05$, 2 way ANOVA followed by Student-Newman-Keuls test for multiple comparisons). In the multiple comparisons, the respiratory and HR responses between 10-50 s were significantly different from tap water control group while the MAP responses were significantly different between 10-40 sec (Fig. 1; $P < 0.05$, 2 way ANOVA followed by Student-Newman-Keuls test for multiple comparisons).

PBG reflex response in PBW group

In this group also, PBG-induced bradycardiac, hypotensive and respiratory responses were

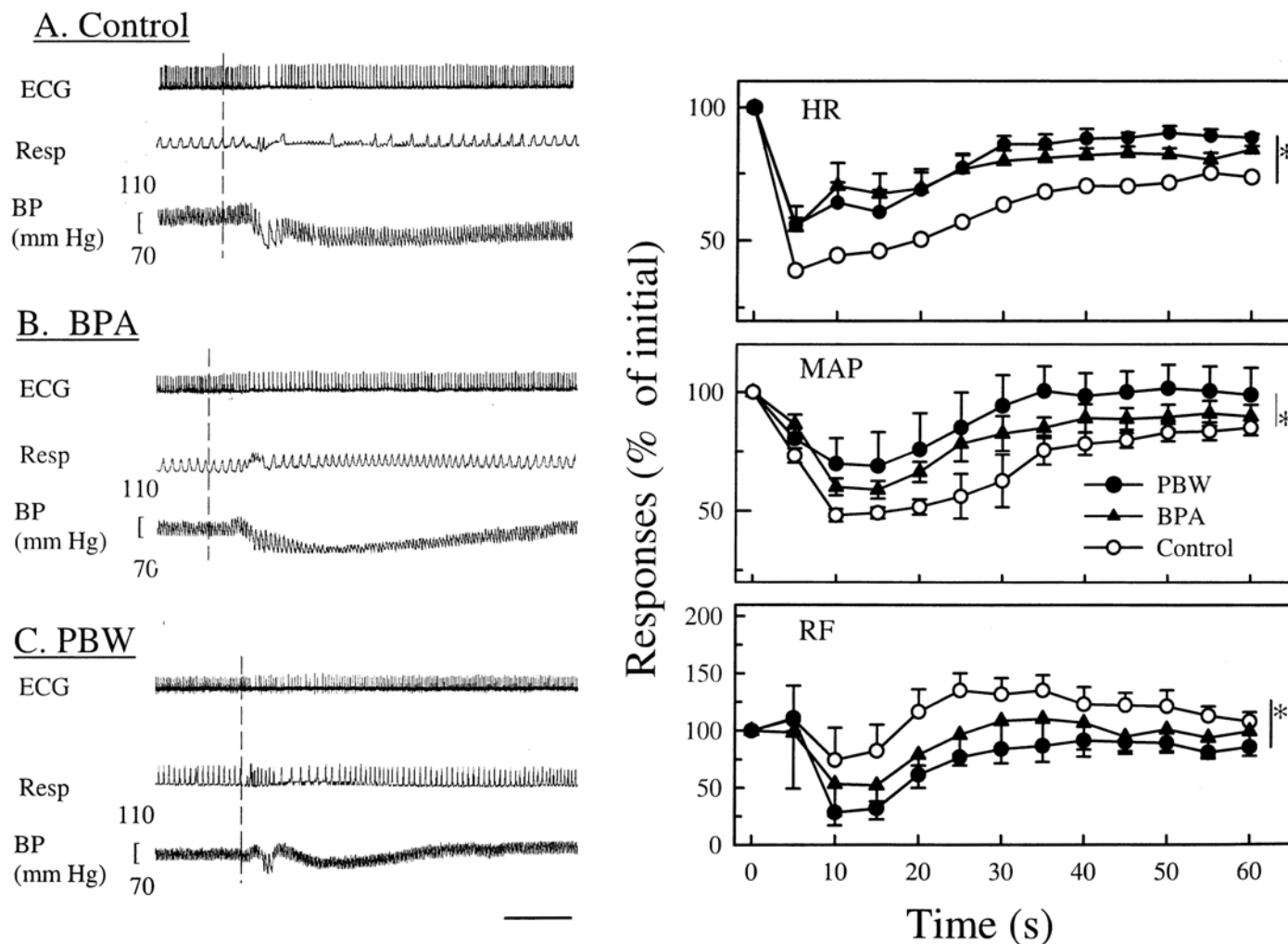


Fig. 1: Chronic exposure to BPA/plastic boiled water (PBW) attenuated the phenylbiguanide (PBG)-induced reflex response. In the left panel, original recordings of PBG response in chronically treated animals with tap water (control), BPA (2 µg/kg/day) and PBW are shown. The dashed lines represent the point of jugular venous injection of PBG (10 µg/kg). The horizontal line in C indicates 5 s (common for all). In the right panel, mean ± SEM responses (as % of initial) at every 5 s are given. The data are from 6 different experiments in each group. An asterisk indicates significant difference from control group ($P < 0.05$; 2 way-ANOVA followed by Student-Newman-Keuls test for multiple comparisons). HR, Heart rate; RF, Respiratory frequency; MAP, Mean arterial pressure; and Resp, Respiratory excursions.

attenuated significantly as compared to control group (Fig. 1; $P < 0.05$, 2 way ANOVA followed by Student-Newman-Keuls test for multiple comparisons). In case of respiratory responses, the tachypnea responses was observed in control group while in PBW group bradypnea response was seen (Fig. 1).

Histopathological changes in lungs

The histopathological examination of lungs in control group receiving tap water revealed normal

cytoarchitecture of lung parenchyma with distinct and clear alveoli (Fig. 2). However, in BPA treated group, patchy areas of consolidation with colloid deposits were observed. In addition, there were mild emphysematous changes in the lung parenchyma and widespread lymphocytic infiltrations (Fig. 2). In PBW treated group, prominent and widespread emphysematous changes were seen in the lungs. In addition, there were areas of colloid deposits and consolidation along with lymphocytic infiltration surrounding the colloid deposits (Fig. 2).

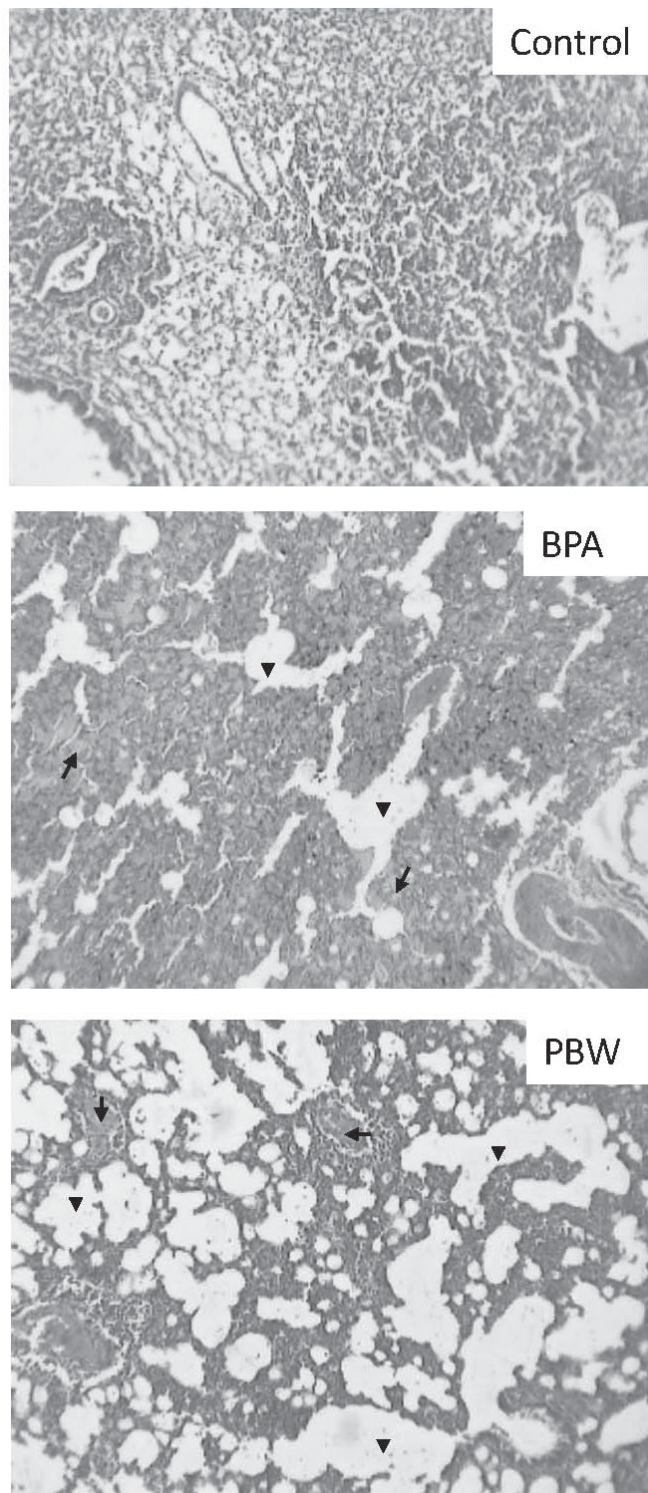
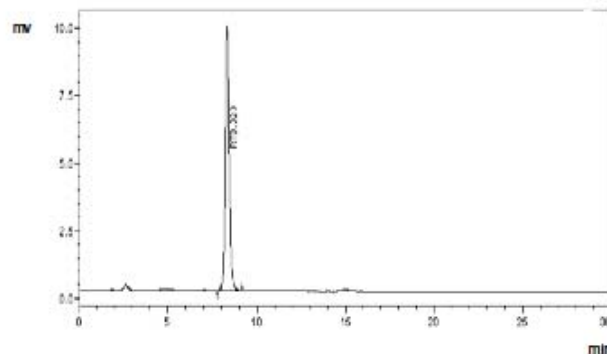


Fig. 2: Photomicrographs of the hematoxylin and eosin stained sections of lungs from rats ingested with tap water (control), BPA (2 $\mu\text{g/kg/day}$) and plastic boiled water (PBW) for 30 days are shown. Note the normal alveolar pattern in control group. Emphysematous changes in alveoli are indicated by arrowheads in both BPA and PBW treated groups. Arrows with tail show the colloidal deposits in BPA and PBW groups. Widespread lymphocytic infiltration is seen in BPA group. In PBW group, the lymphocytic infiltration is seen around the colloid deposits (magnification is 100 \times in all).

A. Standard BPA peak (100 $\mu\text{g/ml}$)



B. Plastic boiled water

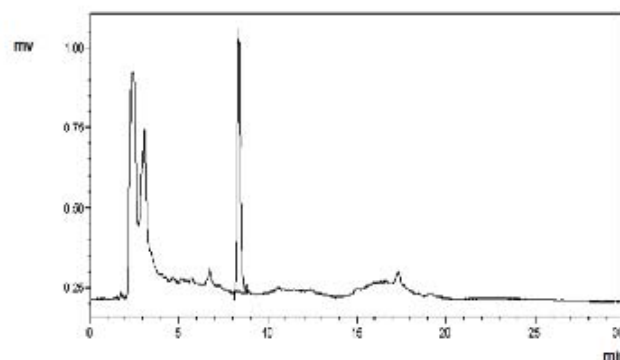


Fig. 3: (A) Chromatogram of 100 ppm of BPA to serve as standard, with retention time of 8.323 min and area of 155971; (B) Chromatogram of PBW clearly showing BPA peak with a retention time of 8.360 min and area of 10310.

Detection of BPA content of PBW

PBW was analyzed through HPLC. A sharp peak was obtained in PBW (Fig. 3). The area and retention time of BPA peak in PBW was comparable to the 100 $\mu\text{g/ml}$ standard peak of BPA. The area obtained in standard chromatogram was 155971, whereas that obtained in PBW was 10310. Hence, this data was used to calculate the amount of BPA in PBW which is equal to 6.6 $\mu\text{g/ml}$ (Fig. 3).

Discussion

The results of this study demonstrate that chronic

ingestion of PBW or BPA in rats produced functional and morphological changes in cardio-respiratory system. The functional changes are seen as decrease in mean arterial pressure (MAP) and attenuation of the PBG-induced cardio-respiratory reflexes as reported earlier for BPA (12). In addition, severe morphological changes were seen as widespread emphysematous, consolidative and infiltrative changes in the lungs.

PBG, a 5-HT₃ receptor agonist, produces cardio-respiratory reflexes. PBG responsive receptors are located in the lungs (pulmonary C or J-receptors) and in the heart around coronary vessels and their stimulation produce pulmonary C reflex/J-reflex or Bezold-Jarisch reflex respectively (17, 18). The response produced by these reflexes manifest as bradycardia, hypotension and apnoea-tachypnoea which serve as protective reflexes in response to lung irritants, pulmonary congestion, myocardial ischemia/congestion, etc (17, 18). In the present study, exposure to PBW attenuated the PBG-induced reflex responses. The attenuation of the reflex can result either from the decreased activity at afferent/efferent nerve terminals or from the suppression of the centers. Earlier we have shown that BPA attenuates PBG-induced reflexes by decreasing the vagal afferent activity (10).

In the present study, the attenuation of the PBG-induced reflexes may have resulted from the gross morphological pulmonary changes induced by BPA present in PBW (Fig. 2). These histological findings resemble the pathological changes observed in lungs with severe degree of emphysema. Emphysema is associated with loss of elastic recoil leading to alveolar inflation (19). In addition, loss of alveolar supporting tissue and narrowing of the airway is also

reported in emphysema (19). These factors favor alveolar dilatation and loss of interalveolar space as seen in the histological study in PBW treated group. The interalveolar space contains number of cellular elements including neural endings (sensory receptors) and type II pneumocytes. Attenuation of reflex response may be due to the loss of sensory receptors in the interstitium. The loss of type II pneumocytes may support for the emphysematous changes observed in the lung pathology (Fig. 2). It is known that type II pneumocytes synthesize surfactants which preserve the alveolar architecture. The decreased surfactant allows smaller alveoli to empty in to large alveoli and increase the alveolar dilatation further (19). Such an effect can be expected in PBW treated group.

Taken together, our observations demonstrate chronic changes induced by PBW/BPA. These changes are probably initiated by acute suppression of protective pulmonary reflexes by BPA to start with. Such suppression decreases alveolar emptying and produce emphysematous changes in the lungs. Alveolar dilatation decreases interstitial space and elastic recoil which further produces alveolar dilatation. In addition, the interstitial cellular loss (type II cells, neural elements) results in decreased surfactant and decreased vagal mediated reflex activity. All these factors finally, produce emphysematous changes in the lungs of animals chronically exposed to BPA.

In conclusion, exposure to PBW and BPA can produce damage to the lung parenchyma/interstitial cells. These changes will further decrease the reflex activity that protects the cardio-respiratory system. Thus, the chronic exposure to plastic leached water produces severe cardio-respiratory abnormalities.

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