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Acute toxic effects of Bisphenol A on *in-vitro* contractile activity of gut in neonatal rats.

Kumari Nirja, Parul Sharma, Anil Kumar Tiwari*, and M B Mandal.

Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

ABSTRACT

Plastic is today an in separable part of daily life. Bisphenol A, a plastic toxin, leaches from the plastic containers and inner coating of metallic containers of food and beverages and contaminates the edibles. Along with food this toxin reaches our body and has been found to produce diverse ill effects on various body systems. The effect of Bisphenol A on gastrointestinal system has yet not been studied thoroughly. The neonatal gut is obviously immature and more likely to be vulnerable to Bisphenol A toxicity. In neonate, gutis exposed to Bisphenol A mainly through lechate from plastic feeding bottles. We therefore attempted to study the effect of Bisphenol A on contractile tension and frequency of contraction of ileum and colon segments of neonatal ratsby *invitro* experiments. Segments of large gut (colon) and small gut (ileum) were dissected out and isometric contractions were recorded in an organ bath preparation using force transducer and digitized data acquisition system. The observations of present study suggested that Bisphenol A decreased the frequency of contraction and contractile tension of ileum and colon significantly ($p<0.05$) in a dose dependent manner and independent of oestrogen receptors, NO and cholinergic system.

Keywords: Bisphenol A, contractile tension, frequency of contraction, neonatal rat, gut

Corresponding author

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INTRODUCTION

Bisphenol A (BPA),chemicallyan organic compound with two phenol functional groups, is used commercially to produce epoxy resins and polycarbonate plastics [1].Epoxy resins form protective internal coating of the metal cans used to store various food items. Polycarbonate plastics are used in making water bottles, baby feeding bottles and other food and beverage containers.BPA leaches from these plasticcontainersinto the edibles.The gastrointestinal systemprimarily, and subsequently other body systems are exposed to BPA during the consumption of thesecontaminatedfood items. Neonatal gut is exposed to BPA through consumption of lechate from these plastic feeding bottles [2].BPA has been detected in various tissues and body fluids of people worldwide [3, 4].Actions of BPA resembleoestrogen[5,6] and its potential health hazards in various body systems have been studied. BPA-induced changes in function of the hypothalamus-pituitary-thyroid axis have been reported [7]. BPA has been reported to produce reproductive and behavioural toxicities [8]depress atrial contractility in rats[9] and affect function of coronary smooth muscles [10].

The main route of exposure of neonate to BPA is oral,so the first system to be exposed to BPA is gut. Some studies have demonstrated that BPA affects gut barrier and immunologicalbehaviour[11, 12] but there is paucity in studies on effect of BPA on intestinal motility. In an observation elsewhere, it has been reported that inhibition of duodenal motility by BPA is mediated by nitric oxide pathway [13]. A recent study in our lab (unpublished observation) suggests that BPA depresses contractile activity in adult rat ileum and colon, in terms of contractile tension aswell as frequency of contraction, *in vitro*.

Neonatal intestine, which is yet to achieve functional maturity, may be more susceptible to ill effects of BPA.The effect of BPA on neonatal intestinal motility is notclear.Since there is no report available to demonstrate the in *vitro* or *in vivo* effects of BPA on neonatal gut contractility, in animals or human beings, the present study was designed to investigate the acute effects of BPA on contractility of neonate rat small gut (ileum) and large gut (colon).

MATERIALS AND METHODS

Animals

Studies were conducted on 1-2 weeks old albino rats of Charles foster strain (either sex) weighing 10-25 gm. Rats were fed *ad libitum* rat feed and potable water, and placed in the animal house of the department with controlled conditions of room temperature (25 ± 0.5^{0}C) and light (12:12hr – light: dark).The present experiments were carried out after the approval of institutional ethical committee for animal experiments.

Dissection of animal

Rats were sacrificed by decapitation. The details of dissection and the procedure for mounting and recording of contractile responses have been described earlier[14,15].Briefly, segments of colon and ileum were dissected out and isometric contractions were recorded in Dale's organ bath with the help of force transducer and digitized data acquisition system. The gut segment was placed under optimum resting tension (0.25 g).

Before, as well as after recording the contractile responses, calibration for the tension (0-10 g) was performed. After stabilization, the initial recordings of spontaneous contractions were made for 30 minutes, without any external chemical intervention. Subsequently, the tissue segment was exposed to different concentrations of BPA (1-100 µM). In experiment using various antagonists like L-NAME (N – nitro – L – arginine methyl ester, a Nitric Oxide synthase inhibitor, Tamoxifen (an oestrogen receptor blocker), Atropine (a muscarinic receptor blocker) and Hexamethonium (a ganglion blocker), the tissue was first treated with respective antagonist, and then the effects of BPA (100 µM) exposure were reassessed.

Drugs and solutions

L NAME, Tamoxifen, Atropine and Hexamethonium were procured from Sigma Chemicals Inc. (St Louis MO, USA) and prepared in double distilled water to have stock solution (10 mM). BPA was obtained from HIMEDIA laboratories Pvt. Ltd, Mumbai and dissolved in 50% ethanol and stock solution (10mM) was

prepared. Tamoxifen was used in 10µM concentration and all other antagonists in 100µM concentration. The stock solution was refrigerated and final dilution was made in the Krebs-Ringer solution. Krebs-Ringer solution was prepared with following compositions (in mmol): NaCl, 119; KCl, 4.7; $CaCl_2.2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 1.2; $NaHCO_3$, 5; and glucose, 11 and pH of the solution was maintained at 7.4. All the chemicals used in the present study were of analytical grade.

EXPERIMENTAL PROTOCOL

In the present study three sets of experiments were designed. After stabilization period of 30 min. control recordings were obtained. In set I, cumulative dose (1, 10, 30 and 100 µM) response of BPA was assessed after exposing the tissue to each dose of BPA for 15min. As ethanol was used as vehicle for BPA, effect of ethanol (equi. volume in respective BPA concentrations) was assessed in set II. To find, if the effect of BPA could be blocked by any of the four antagonists, the set III was divided in four subsets and the gut tissue was exposed to one of four antagonists in one of these subsets for 15 min & subsequently it was exposed to BPA at concentration of 100 µM for 15 min.

At the end of each experiment the tissue segment was blotted to remove extra water and weighed.

Parameters assessed in study and their statistical analysis

Contractile tension and frequency of contractions were studied. The contractile tension per unit mass of tissue (g/g wet tissue) was calculated and the values were pooled to express as mean± SEM and % of initial tension. One or two-way ANOVA was applied for multiple comparison as appropriate. Paired and unpaired *t*-test as applicable was employed to determine the statistical significance of results obtained. The tests were considered significance when *p*-value was <0.05.

RESULTS

Features of contractions in control recordings

The control contractions were of mixed type i.e. tonic contractions with superimposed phasic contractions in both ileum and colon. Table 1 describes the mean ± SEM values of spontaneous contractions and frequency of contractions in control samples of ileum and colon as observed after stabilization period of 30 min. The control values of phasic frequency (contractions/sec was significantly different in ileum and colon of neonatal rat, as reported earlier (15).

TABLE 1: Mean ±SEM values of spontaneous contractions expressed as g/g wet tissue and frequency of contractions/unit time in ileum and colon of neonate rats in control samples. Asterisk indicates significantly different value compared to ileum (unpaired t test)

Parameters	Untreated samples	
	Ileum	Colon
Contractile tension (g/g wet tissue)	8.88±1.67	6.61±0.29
Frequency (contractions/min (tonic)	10.25± 2.66	12.75±0.95
Frequency (contractions/sec (phasic)	18.17± 0.98	9.5±1.94*

Cumulative dose (1-100 µM)response of BPA

Both ileum and colon tissue demonstrated dose dependent decline in the amplitude of contractions. In the present study there was significant ($p<0.05$, one way ANOVA) decrement in the contractile response/tone of contractions on increasing the dose of BPA. The response of highest dose (100 µM) of BPA was 47 % of initial in ileum (Fig. 1a) and 57% of initial in colon tissue (Fig. 2a). At 30 and 100 µM of BPA concentrations (Fig. 1b, 2b)there was a significant decrease in tonic frequency and the frequency became zero at 100 µM BPA but few phasic (2.25/ sec) contractions were observed in both ileum as well as colon tissue.

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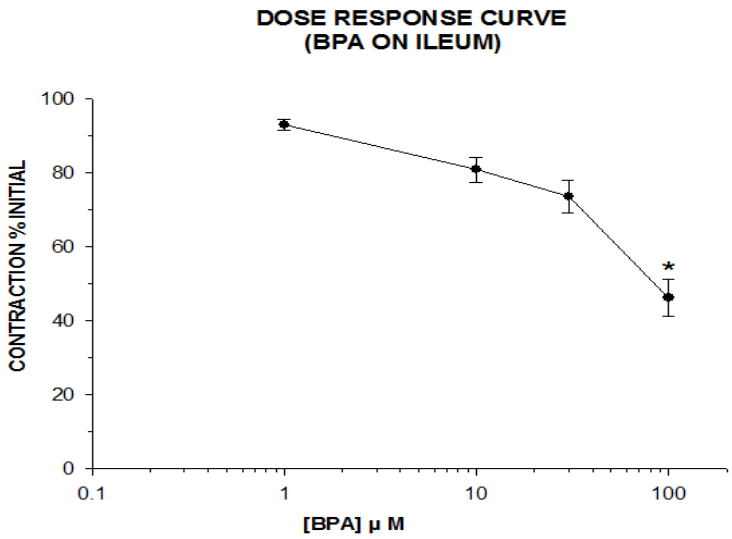
(a)



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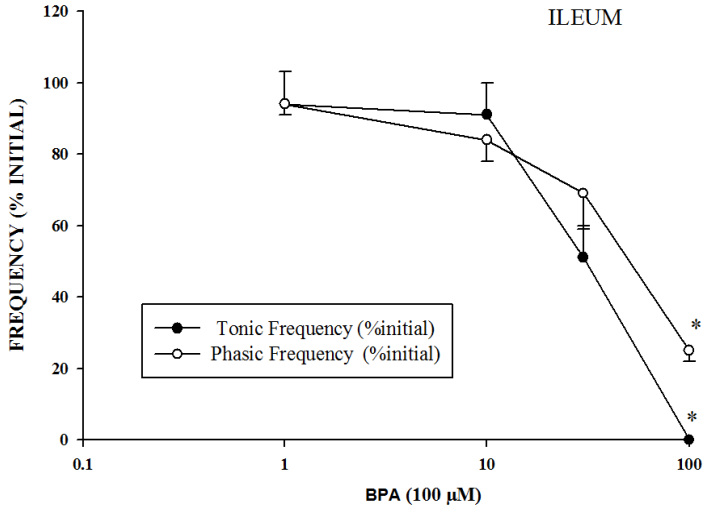

Fig 1: Graphs demonstrating the dose response of BPA on (a) contractile tension (b) and frequency (tonic and phasic) of contraction per unit time (data points represent Mean ± SEM values, n= 4) in neonate ileum. The asterisk indicate significantly (p-value <0.05, one way ANOVA) different values from previous values.

Ethanol was used as vehicle for BPA and different concentration of ethanol used to dissolve respective concentrations of BPA could not produce any significant alterations in contractile response/tone as well as frequency of contractions(p-value >0.05, one way ANOVA). Thus, ethanol per se did not have any significant effect on contractile parameters (communicated for publication)

BPA (100 µM) response in antagonists pre-treated ileum and colon tissue

There was no significant (p> 0.05) difference between the response of BPA (100µM) with or without pretreatment of antagonists (fig.3a, 3b). In ileum (fig.3a) as well as colon (fig.3b)pre-treatment with all the antagonists failed to block BPA (100 µM) induced diminution in contractile responses, The frequency was considered zero after the addition of BPA 100 µM as the frequency of contraction was not recordable (due to flatted record) in all the pre-treated groups.

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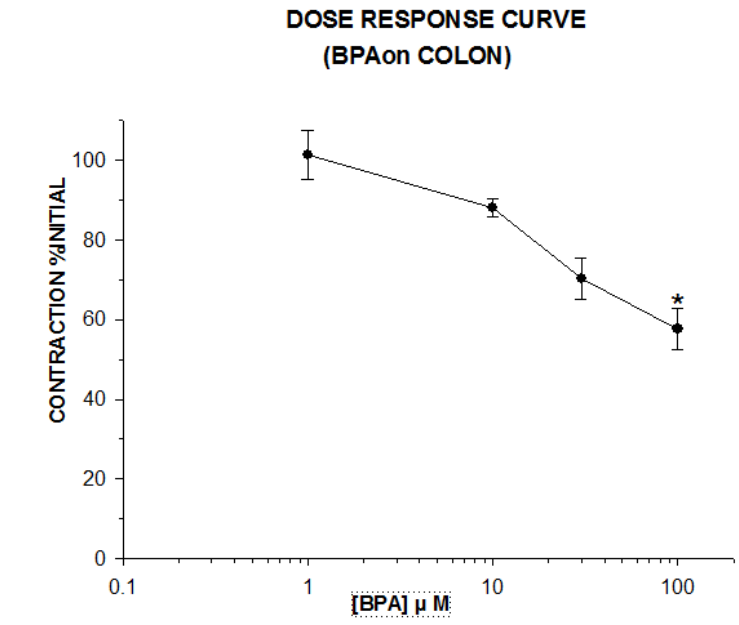
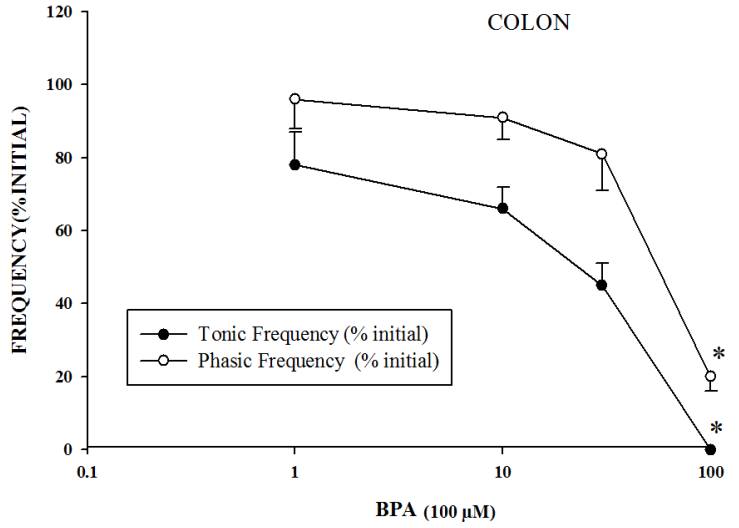


(a)



(b)

Fig 2: Graphs demonstrating the dose response of BPA on (a) contractile tension (b) and frequency (tonic and phasic) of contraction per unit time (data points represent Mean ± SEM values, n= 4) in neonate colon. The asterisk indicate significantly (p-value <0.05, one way ANOVA) different values from previous values.

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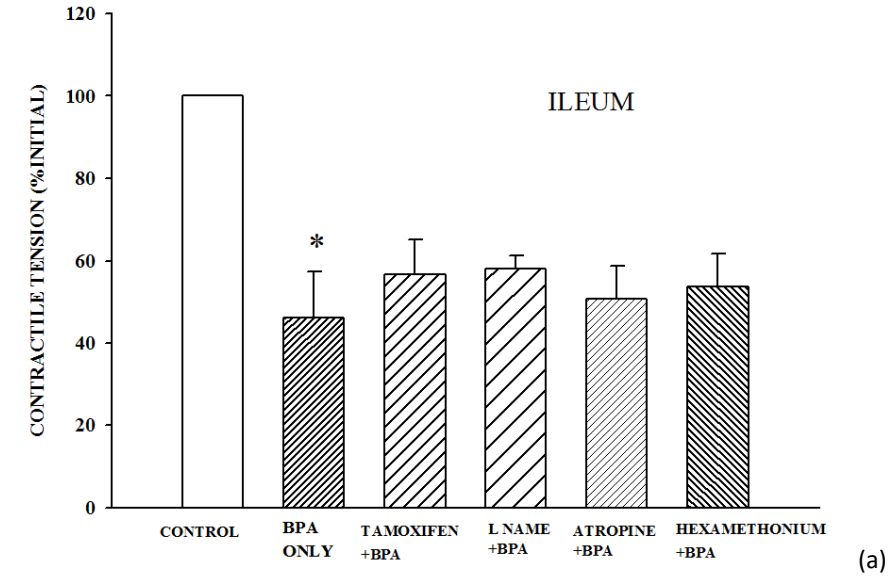

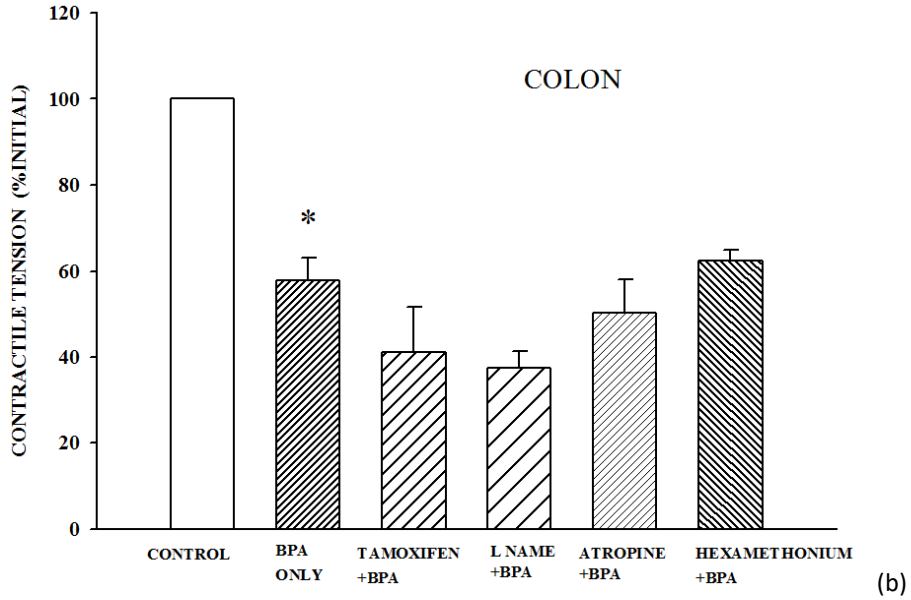

Fig 3: Bar diagrams showing (Mean± SEM, n=4) the contractile tension in (a) ileum and (b) colon after addition of BPA only (100 µM) and addition of BPA after treatment with Tamoxifen (10 µM), L-NAME(100 µM), Atropine (100 µM) and Hexamethonium (100µM), expressed as percentage of initial. *(p-value <0.05) as compared to control (n=4, paired *t test*). There was no significant (p-value >0.05) difference between BPA only and BPA+ any of the antagonist groups.

DISCUSSION

In the present investigation BPA decreased both contractile tension and frequency of spontaneously occurring contractions in ileum as well as colon segments of neonatal rats. The amount of ethanol used for dissolving various concentration of BPA did not alter the smooth muscle contractile activity significantly, therefore it was concluded that the observed inhibition of contractility was due to BPA and not the vehicle (i.e. ethanol). The attenuation of contractility was demonstrated by diminution of both contractile tension and frequency. Reduced tension generated in intestinal smooth muscle may be ascribed to effects of BPA on contractile machineries and observed changes in the frequency of contractions may be attributed to influence of BPA on interstitial cells of Cajal.

It has been reported that actions of BPA are similar to oestrogen [16] and oestrogen impairs contractile activity of gut muscle [17]. Therefore, the oestrogen mimicking activity of BPA may be responsible for decreased contractile functions of gut observed in the present experiments. There are two known receptors of estrogen (ER), namely ERα and ERβ [18]. The ERβ has been reported to be present in intestine [19]. However, in present investigation the BPA induced inhibitory response was not blocked by oestrogen receptor antagonist tamoxifen. This signifies that the action of BPA in ileum and colon might be independent of ER.

Further, BPA has been found to depress the atrial activity through nitric oxide (NO) mechanisms [9]. Involvement of NO mechanisms in this study was assessed using L NAME(nitric oxide synthase inhibitor) but it was found that pretreatment of L-NAME failed to shield BPA-induced diminution in contractility indicating non-involvement of NO mechanisms. However in an observation elsewhere [13] BPA has been found to inhibit the movement of the duodenum through NO mediated mechanisms.

It was hypothesize that BPA might mediate its action via neural elements in enteric nervous system. However, this could not be verified in our experiments using ganglion blocker Hexamethonium. Also non involvement of cholinergic mechanisms was verified by experiments having pre-treatment with atropine.

Therefore, it is apparent that the diminution in contractile response demonstrated by BPA in this study was mediated by its ER independent action on intestinal smooth muscle. Further, studies elsewhere suggest actions of oestrogen mediated by activation of potassium channels or inhibition of calcium channel, without involvement of ER receptors [20]. However, in the present investigation the operation of similar machanisms could not be verified.

To summarise, BPA reduced the contractility ofileum and colon segments without involvement of oestrogen receptors, nitric oxide and intrinsic neural plexuses. The present experiments cannot explain the precise mechanism behind these observations. Further altered contractile function of ileum and colon produced by BPA in this study may have clinical implications in constipation and other intestinal motility disorders, hence additional detailed investigation is required.

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