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EFFECT OF ASPHYXIA ON BLOOD PRESSURE AND URINE FLOW IN NICOTINIZED ANIMALS: ROLE OF SYMPATHETIC ADRENOCEPTORS

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ABSTRACT ■ In the present study attempts have been made to study the combined effects of experimentally induced asphyxia and nicotine alterations in cardio-renal functions of cat. At the same time attempts have been made to find out the possible sympathetic adrenoceptors involvement in asphyxia along with nicotine-induced alterations in hypertension as well as urine flow. It was noted from the results that asphyxia caused hypertension associated with changes in urine flow (antidiuresis and diuresis). These cardio-renal changes were significantly more profound in case of asphyxia along with the nicotine. Pretreatments with specific sympathetic blocker, asphyxiated and asphyxia with nicotinized animals, failed to elicit any alterations in blood pressure; but at the same time antidiuresis and diuresis were partially counteracted in such animals. It may be concluded that catecholamines released by asphyxia and nicotine may cause initial rise in blood pressure along with antidiuresis, which are the effect of á adrenoceptors involvement. Subsequent diuresis during falling phase of blood pressure is not related with α adrenoceptors. The role of α adrenoceptors is greater during asphyxia along with nicotinized condition in comparison to asphyxia alone.

Key Word: Asphyxia, Nicotine, Hypertension, Urine Flow, Sympathetic adrenoceptors.

INTRODUCTION

The features of asphyxia are hypercapnia (increased levels of carbon dioxide in blood), hypoxemia (low oxygen concentration in arterial blood), and ischemia (diminished amount of blood perfusing the brain) (Volpe, 2001). Respiratory diseases, including chronic obstructive pulmonary disease (COPD) and lung cancer, account for a large proportion of tobacco-related deaths. All types of COPD or other lung dysfunctions can cause asphyxic condition. At the same time WHO considers smoking to be the leading preventable cause of death worldwide and a chronic recurrent disease caused by nicotine dependence (ND) (WHO, 2011). Most of the smokers are unaware of the damage caused by chronic tobacco use, and nearly half will die of a tobacco-related disease (WHO, 2011). A complex behaviour, ND is influenced by genetic, social, and environmental factors, and is therefore considered a chronic cardiovascular and renal disease that requires repeated interventions (Chatkin, 2006;

Reichert et al., 2008 and Henningfield et al., 2009). Tobacco is the most important risk factor for cancer and more than 50 diseases. Continuing to smoke after a diagnosis of COPD or asphyxia related conditions, contributes to a higher risk of cardio-renal complications during treatment as well as leading to worsening of other tobacco-related diseases (Chen et al., 2011; Pinto et al., 2011 and Toll et al., 2013). Cardio-renal dysfunctions contribute to increase the morbidity and mortality rate during the time of smoking with COPD. Maintaining tobacco use along with COPD increases the risk of recurrence with decreased quality of life and overall survival.

In asphyxia the sympathetic centre in hypothalamus is activated, whereas the parasympathetic centre is depressed. The Sino-aortic chemoreceptors are not related to the activation of the sympathetic centre in hypothalamus during asphyxia and it is initiated in asphyxia through direct action of the changes in the blood gases on the autonomic central structure. Vagal effect on pressor response during asphyxia came later and sympathetic activity was presumably the major effect on such pressor response (Ghosh and Koley, 1977). The acute response to hypocapnic hypoxia (asphyxia) is well characterized as exposure of healthy humans causes systemic vasodilation, pulmonary vasoconstriction, heart rate (HR) mediated increases in cardiac output and variable blood pressure responses during the exposure (Blitzer et al., 1996a,b). At the start of a severe hypoxic challenge, a rapid, and largely chemoreflex-mediated (Bartelds et al., 1993), activation of the sympathetic nervous system (SNS) leads to peripheral vasoconstriction that helps redirect blood flow to critical organs, such as the heart and adrenals (Giussani et al., 1993 and Wassink et al., 2007). Koley and Mukherjee (1964) suggested that asphyxic vasopressor response in intact cat was due to sympathetic and sympathoadrenal activation. During asphyxia, vasopressor response in intact cats, is presumably occurring through the release of the adreno-medullary hormone, norepinephrine (NE). But Borovsky and their group (1998) pointed out that the increase in plasma NE was associated with sympathetic nerve ending release and not adreno-medullary release, was mainly due to hypoxia, and was not a specific response to CO_2. They reported that adrenalectomy did not significantly reduce the NE response to CO_2. Ghosh and their co-workers (1977) reported that asphyxic pressor response was due to excitation of a-adrenergic receptors. β-adrenoceptors have a little role on such pressure response. The mechanism of this vasoconstriction involves α adrenergic receptor stimulation mediated by both cardio-sympathetic nerves and circulating catecholamines. Moderate hypoxia in the near-term fetus is associated with an acute decrease in fetal urine production (Robillard et al., 1981), whereas during prolonged hypoxia urine output and glomerular filtration rate (GFR) and urine osmolality rapidly normalize (Cock et al., 1997). Similarly, in near-term fetal sheep prolonged moderate hypoxia was associated with an increase in renal blood flow, which returned to control values during recovery (Braaksma et al., 1999 and Green et al., 1997).

Nicotine has been considered to be one

of the causes of hypertension by the activation of the peripheral sympathetic nerve endings resulting increased release of catecholamines (Koley et al., 1987b; Marano et al., 1999) and also by activating the central nicotinic receptor sites; resulting release of catecholamines both from the adrenal medulla and adrenergic nerve terminals (Kubo and Misu, 1981; Mayhan, 1999). This hypertension is always associated with the altered in renal functions. The adrenal release of catecholamines is primarily responsible for increased myocardial contractile function during intravenous nicotine administration (Downey, 1990). The effects of smoking as well as the nicotine affects on sympathetic nervous system and on systemic hemodynamics accompanied by significant acute changes in renal hemodynamics (Ritz et al., 1998). Cigarette smoke-induced renal damage is due, at least in part, to the activation of sympathetic nervous system (Odoni et al., 2002). Neurotransmitters released from the renal sympathetic nerve endings play a central role in the regulation of Na^+ excretion (Bello-Reuses et al., 1975; DiBona, 1985). NE activates both a and b adrenergic receptors. Activation of a-adrenergic receptor enhances tubular sodium reabsorption (DiBona, 1985) and stimulates the activation of proximal tubular Na^+-K^+-ATPase (Meister et al., 1994) and b-adrenergic receptors are positively coupled to adenylate cyclase (Hanson & Linas, 1995) and control the tubular function. Haldar and his group (2001) reported that nicotine can cause the alteration in urine flow (antidiuresis and diuresis) through adrenergic neurotransmitters.

Only limited data on the effect of asphyxia with nicotine induced alterations in blood pressure and urine flow are available in the literature. These data suggest that sympathetic and adreno-medullary responses to asphyxia along with nicotine in case of cardio-renal changes may be immature. Potentially, this immaturity may compromise the ability of the animals to maintain homeostasis during severe hypoxic stress during COPD; however, there are few direct data in this field. The aim of the current study was to found whether nicotine (nicotine of smokers' blood) could aggravate further the cardio-renal changes during asphyxia or not. At the same time effort will be made to find out the specific role of sympathetic adrenoceptors (α or α) behind this hypertension and impaired renal tubular function.

METHODS AND MATERIALS

1) Experimental design:

Twenty adult cats of either sex weighing between 2-3 Kg were studied. All the animals were classified into two groups consisting of ten animals in each group. Group- I represented the asphyxiated animals without nicotine drip and Group-II represented asphyxiated animals along with nicotine drip. In these two groups of animals drugs were applied.

2) Animal preparation:

The investigation was carried out on normal adult cats of either sex weighing between 2-3 Kg and maintained with nutritious food and water. The day before the experiment the cats were given water *ad libitum* and no solid food was given. The rectal temperature was noted by using a thermometer (Zeal, UK) and the temperature (37°± 0.5°C) was maintained throughout the experiment using the

heating pad placed below the operating table. The cats were anaesthetized by injecting á-chloralose (60-70 mg / Kg. body weight; i.v.) through femoral vein after an initial induction with anesthetic ether and the á-chloralose was maintained throughout the experiment with a maintenance dose of 10 mg / Kg. Body weight (i.v) when required. The experimental protocols were according to the guidelines of International Ethical Committee (Registration No. 506/01/a/ CPCSEA).

3) General surgical preparation before the experiment:

A portion of the skin was cut off over the femoral vein at the junction of body and right hind leg. Then the femoral vein was cleared off from the surrounding tissues. An incision was made over the femoral vein and a polyethylene tube, filled with normal saline, fitted to a three-way stopcock (Pharmaseal, U.S.A.) at one end and other end was introduced to the femoral vein for administration of drugs and saline. In the same way femoral artery of the same side was cleared off from the surrounding tissues and cannulated with another polyethylene tube, also filled with normal saline and fitted with stopcock for recording of blood pressure. Right femoral artery was cannulated for recording of blood pressure through INCO pressure transducer coupled with INCO Polyrite (Koley et al., 1987b).

Artificial ventilation and asphyxia was achieved *via* tracheal intubation. For this intubation an incision was made carefully over the skin and then with the blunt scissor trachea was exposed after cutting the smooth muscle around the trachea. After giving a lateral small incision one end of a 'T' shaped glass tube was inserted in the trachea and tied with a cotton thread firmly.

Left ureter was approached by retroperitonial incision over the left side of lower abdomen. The ureter was cleared off carefully from the surrounding tissues. A very fine soft polyethylene tube was introduced through the ureter after giving an inclined incision. The catheter was pushed upward until the tip was at the opening of the pelvis and fixed by tying with a silk thread. After catheterization, the skin and the smooth muscle over the incision were stitched by sewing, keeping the opened end of the catheter outside the body. The left ureter was cannulated for recording of urine flow as one spike per drop through a drop recorder connected with the INCO Polyrite. The urine flow was calculated as drops/min. (Koley et al., 2001; Haldar et al., 2001). Urethra was exposed ventrally by a small incision over the skin just above the pelvic girdle. Then the urethra was pulled up and one end of a wide polyethylene tube was introduced through the urethra and another end of the catheter was fixed to a three-way stopcock (Pharmaseal, U.S.A.) so that the bladder could be evacuated time to time.

4) Methods of experimental asphyxia and artificial respiration:

Asphyxia was induced experimentally by clamping the free end of the tracheal tube, through which the animal was allowed to respire. Clamping was done during the inspiratory phase and continued for 40 to 90 seconds, if the condition of the animal permitted (Koley and Mukherjee., 1964 and Ghosh and Koley, 1977). In case of animals, which

were artificially ventilated (through artificial ventilator machine), asphyxiation was done only by withdrawal of the ventilation (Koley and Mukherjee, 1964).

5) Administration of Drugs:

All the drugs were dissolved or diluted in the normal saline solution (0.9gm% NaCl) freshly prepared before the experiments. Desired quantities of tested drugs were introduced through the three-way stopcock attached with the femoral vein catheter in all the cases. The infusion of each drug was followed by 0.5 ml. of normal saline.

In all the experiments, the animals were given 5% dextrose saline by drip fed for the maintenance of normal body fluid and electrolyte balance. Femoral arterial blood pH was checked and maintained at normal range either by alteration of ventilation or by infusion of $NaHCO_3$ (8.4%) intravenously.

In the present experimental study nicotine (drip, 8-10 drop/min.) was used intravenously through the right femoral vein in a dose ranging from 20-60 çgm/ Kg. The dose was given on the basis of the fact that in cats on administration of nicotine of 10-20 µgm/ Kg body weight/ min intravenously, the plasma level of nicotine (Gebber 1969; Zapata et al 1976a) was about 40-70 ηgm/ml, which was approximately similar to smoker's nicotine level in blood.

The following drugs which are used intravenously (i.v) in this experiment were very much specific for their action. Guanethidine Sulfate (15mg /Kg. Body weight) was a complete sympathetic blocker. Phentolamine mesylate (2.5mg / Kg Body weight), Prazosin hydrochloride (0.8 mg/Kg body wt i.v.), Yohimbine hydrochloride (0.8 mg/Kg body weight, i.v.) and d, 1 Propranolol hydrochloride (0.5 mg/ Kg body weight) were specific sympathetic antagonist for α-nonselective sympathetic blocker, $\alpha_1$selective sympathetic blocker, α_2-nonselective sympathetic blocker and â-nonselective sympathetic blocker respectively.

The followings are the details of the drugs used:

Anaesthetic ether (Kabra Drugs Ltd., India), Alpha-chloralose (Koch-Light Lab. Ltd. England), Sodium Chloride (E. Merck Ltd., India), Dextrose anhydrous GR (Loba Chemie, India), Heparin (Biological E Ltd. India), Nicotine (Technical, BDH Chemicals Ltd. Pool, England), Guanethidine Sulfate [Sympathetic blocker], Phentolamine mesylate [á-nonselective sympathetic blocker] (Ciba-Geigy, Switzerland), Prazosin hydrochloride [α_1-selective sympathetic blocker], Yohimbine hydrochloride [α_2-nonselective sympathetic blocker] (Sigma, USA), d, 1 Propranolol hydrochloride [α-nonselective sympathetic blocker] (ICI, India).

6) Data Analysis:

The formula was used to calculate the mean arterial blood pressure (MABP) was MABP = DP + 1/3 (SP-DP), where DP was diastolic pressure, SP was systolic pressure. At the same time urine flow was measured by drops / min. One spike indicated one drop of urine.

All data were presented as means ± standard error of mean (SEM). Percent changes in parameters in response to asphyxia and asphyxia with nicotine were calculated using the following formula: (Response value – Control value) / (Control value) × 100. Changes in all

data were analyzed using Student's paired *t*-tests with statistical significance set at P <0.05.

RESULTS

1. Effects of asphyxia on blood pressure and urine flow in normal and nicotinized animals.

The animals were initially allowed to breathe spontaneously. For practical purposes the start of asphyxia has been taken for 40-90 seconds as the condition of the animal was permitted. Initially for a short period, there was no alteration of mean arterial blood pressure (MABP) and urine flow (UF) with asphyxia. Just after release of asphyxia there was a rapid increase in blood pressure. Blood pressure came back to initial level slowly over a period of 5-10 minutes and simultaneously urine flow was also returned to its initial level (Fig. 1).

During asphyxia the average resting MABP was increased by 25.64 % (from 85.85 ± 1.93 mmHg to 107± 2.54 mmHg, *P <0.001*) and changes in the urine flow as antidiuresis was 39.16 % (from 2.86 ± 0.14 to 1.74 ± 0.10 drops / min, *P <0.01*) and diuresis was 66.78% (from 2.86 ± 0.14 to 4.77 ± 0.33 drops / min, *P <0.05*) as shown in Figs. 1 & 2. In this study it was also observed that the asphyxia induced average % change in MABP and UF during antidiuresis (AD) and diuresis (D) were 20.81 ± 1.33 mmHg, -39.57 ± 1.42 drops / min and 35.38 ± 2.43 drops / min respectively (Fig. 3 A & 4 A).

After the intravenous application of nicotine drip (10-20 μgm/kg/min) the blood pressure began to rise slowly and stabilized at a constant level after 20-30

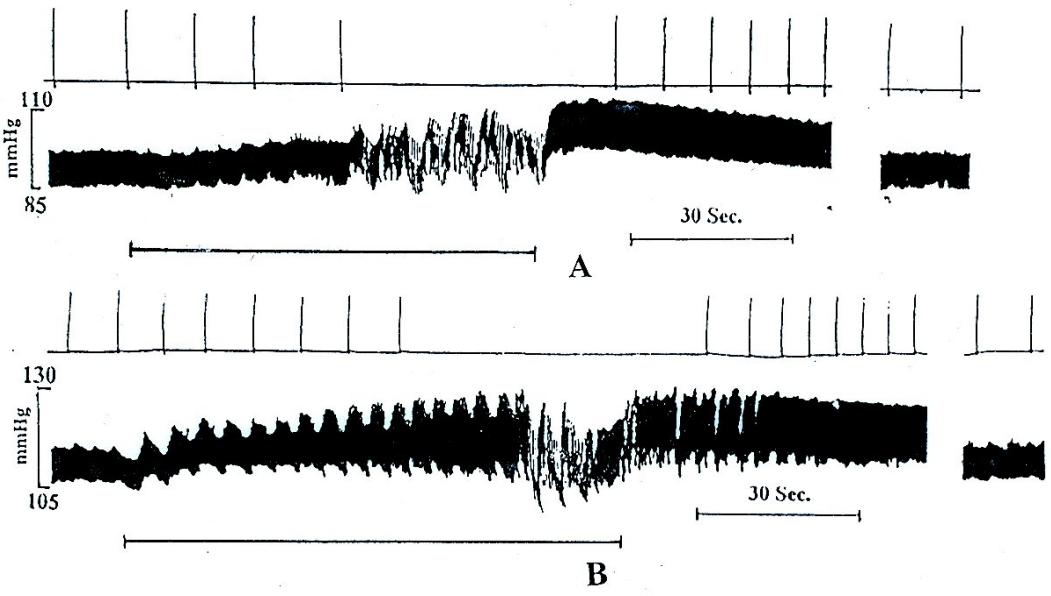

Fig.1. Typical response pattern of blood pressure and urine flow to **asphyxia (A)** and **asphyxia with nicotine (drip) (B).** The upper tracing shows the urine flow and lower tracing shows the blood pressure changes. Each upward spike indicates one drop of urine. The horizontal bar indicates duration of asphyxia. To accommodate the tracing a break for 5 min. is made for each panel.

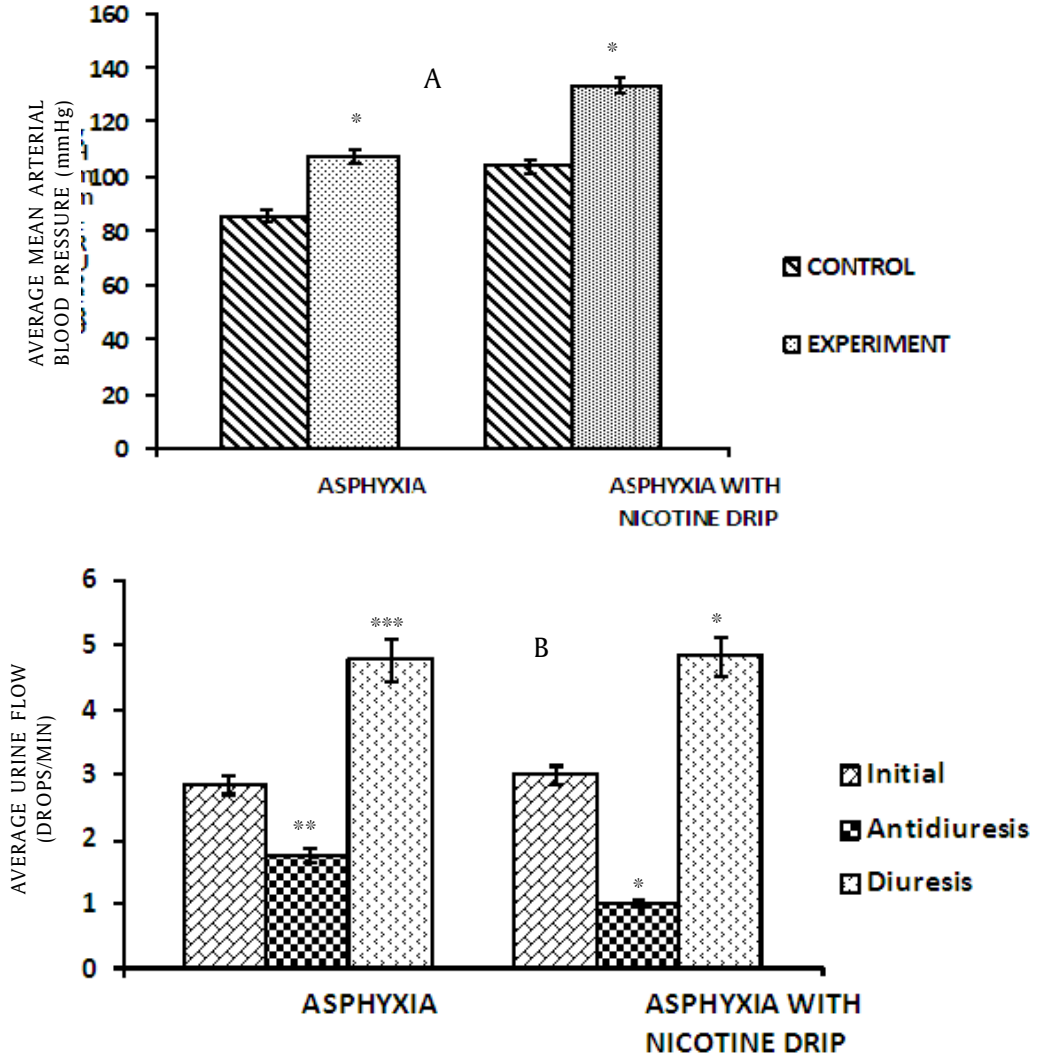

Fig.2: Effects of asphyxia and asphyxia with nicotine drip on cardio-renal changes. Bar graphs showing the **average mean arterial blood pressure (mmHg)** in control (n= 36) and experimental animals (n= 37) (**A: Upper panel**) and **urine flow (drops/min)** as initial (n=28), antidiuresis (n=29) and diuresis (n= 27) (**B: Lower panel**). Values are means ?± SEM $^{*}P < 0.001$; $^{**}P < 0.10$ and $^{***}P < 0.05$ compared with control.

min. When the pressure began to rise, urine flow was also decreased considerably (Fig.1). Due to asphyxia (40-90 seconds) the resting pressure was further increased by 28.84 % (from 104 ± 2.37 to 134 ± 2.67 mmHg, $P < 0.001$) along with more amount of AD (from 3.01 ± 0.14 to 1.00 ± 0.06, drops / min, $P < 0.001$). After withdrawal of asphyxiation as usual post asphyxial rise of pressure was observed, and UF remained in decreased state. But when the BP began to fall UF began to rise (from 3.01 ± 0.14 to 4.83 ± 0.31 drops

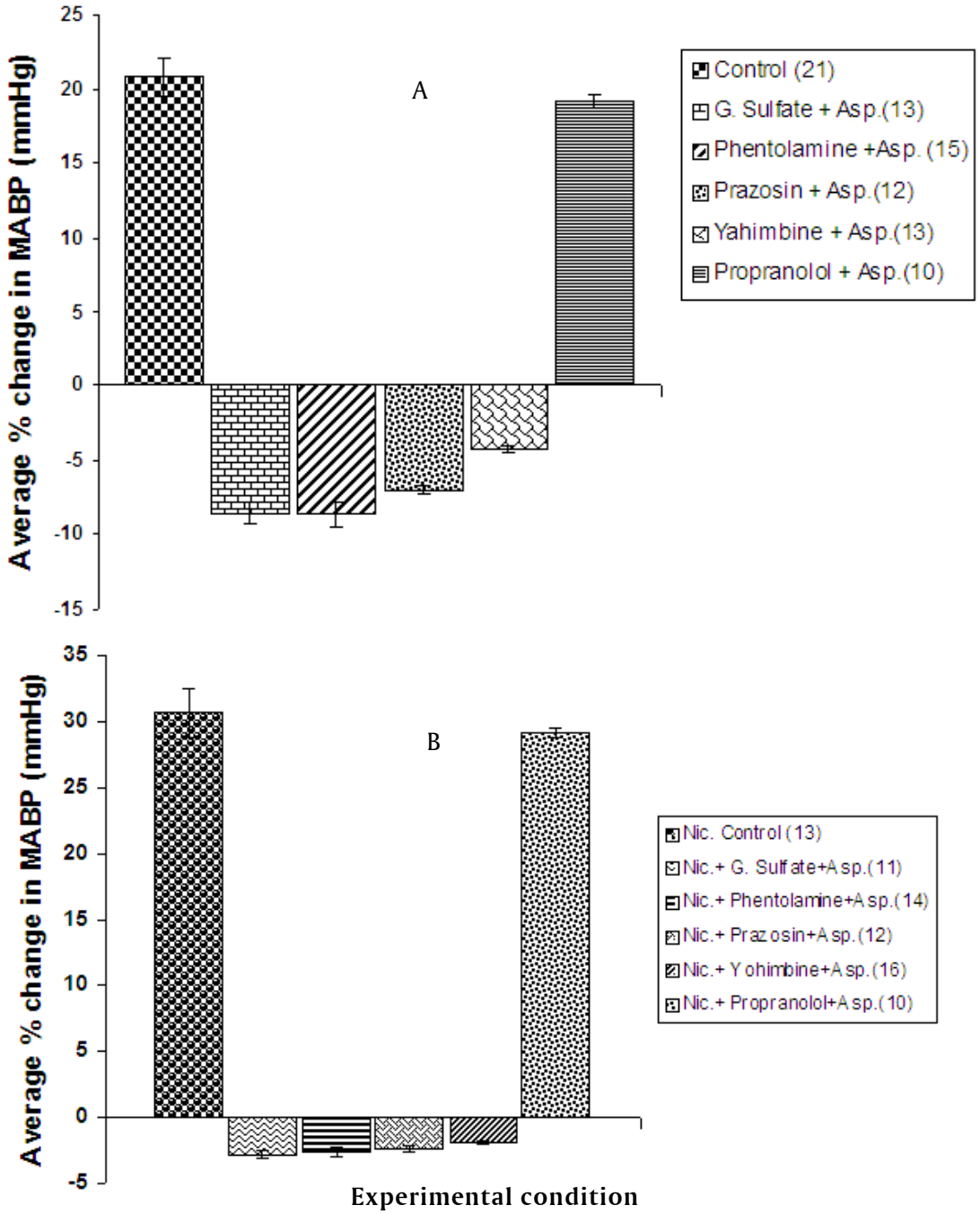

Fig.3. Effects of sympathetic blockers on hypertension. Bar graphs illustrating the average % change in mean arterial blood pressure (mmHg) during **asphyxia (A: Upper panel)** and **asphyxia with nicotine drip (B: Lower panel)** in different experimental conditions. Number with in the parenthesis indicates the number of observations (n). Values are means ± SEM, $^{*}P < 0.001$ compared with control.

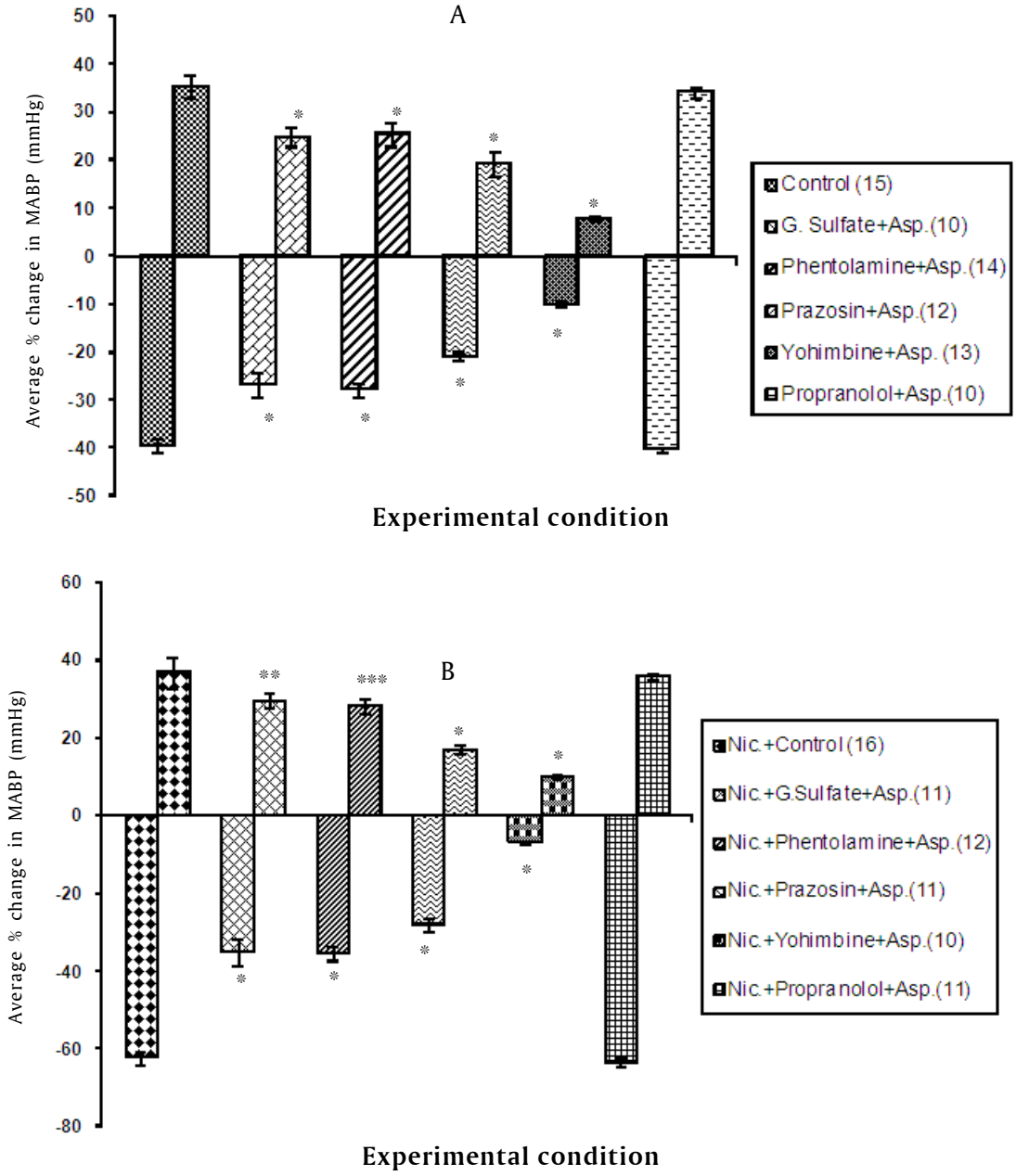

Fig.4. Effects of sympathetic blockers on renal functions like urine flow. Bar graphs illustrating the average % change in **urine flow (drops /min)** during **asphyxia (A: Upper panel)** and **asphyxia with nicotine drip (B: Lower panel)** in different experimental conditions. Number with in the parenthesis indicates the number of observations (n). Values are means ?± SEM $^{*}P < 0.001$; $^{**}P < 0.10$ and $^{***}P < 0.05$ compared with control.

/ min, *P <0.001*) as presented in Fig. 1B & 2B. All these cardio-renal changes were more aggravated than the asphyxia induced alterations only. These results were statistically significant. On the other hand the average % change in MABP during asphyxia along with nicotine (drip) was 30.60 ± 1.85 mmHg and the amount of antidiuresis and diuresis was –62.36 ± 1.6 and 36.66 ± 3.87 drops / min respectively (Fig. 3B & 4B). Thus it was clear that in nicotinized condition asphyxic effects on hypertension and urine flow were more profound and intensive (Fig.1B). However, there was no significant % change in diuresis in nicotinized state in comparison to the results of asphyxia without nicotine.

2. Effect of asphyxia and asphyxia with nicotine (drip) induced blood pressure and urine flow in guanethidine sulfate (GS) pretreated animals.

To study the involvement of sympathetic adrenoceptors, the effect of asphyxia and asphyxia along with nicotine (drip) induced blood pressure and urine flow were tested in animals pretreated with Guanethidine Sulfate (Sympathetic blocker) at a dose of 15mg /Kg. body weight. In such experiments asphyxia and asphyxia with nicotine failed to elicit any alterations in blood pressure. But at the same time AD and D were partially counteracted in such animals (Fig.3 & 4). In guanethidine sulfate pretreated animals the average % change in hypertension to hypotension during asphyxia was 20.81 ± 1.33 to -8.55 ± 0.77 mmHg, (Fig.3A) and antidiuresis was –39.57 ± 1.42 to –26.75 ± 2.42 drops / min and diuresis was 35.38 ± 2.43 to 24.95 ± 1.93 drops / min (Fig. 4A). The above changes were statistically significant (*P<0.001*).

On the other hand in combination with nicotine drip and guanethidine sulfate pretreated animal, asphyxia caused a change towards hypotension (–2.81% ± 0.30, *P<0.001*) instead of hypertension (30.60% ± 1.85) and % changes in urine flow as antidiuresis was from –62.36 ± 1.60 drops / min to –35.10 ± 3.44 drops / min (*P<0.001*) and diuresis was from 36.66 ± 3.87 to 29.65 ± 1.99 drops / min (*P<0.01*) respectively (Figs. 3B & 4B). The above results indicate that adrenergic neurotransmitters (i.e., NE) released from the sympathetic nerve terminals are involved in the asphyxia-induced alterations in hypertension and are partially involved in urine flow in control and nicotinized animals.

3. Effect of asphyxia and asphyxia with nicotine (drip) on blood pressure and urine flow in animals pretreated with phentolamine.

To specify the involvement of adrenoceptors, phentolamine (a non-selective adrenoceptors blocker) was administered intravenously at a dose of 2.5mg / Kg Body wt. In phentolamine pretreated animals the average % change in MABP during asphyxia was altered (Fig.3). The hypertension (20.81 % ± 1.33 mmHg) of control animals were converted to hypotension (–8.61% ± 0.80 mmHg, P<0.001). The reduction in % change from control in this group was highly significant. In such animals asphyxia induced urine flow were counteracted partially but significantly and the average % changes in antidiuresis and diuresis were –27.93 ± 1.35 drops / min (P<0.001) and 25.42 ± 2.62 drops / min (P<0.001) respectively (Fig.4) in comparison to the control value as in

case of GS (Figs. 3A & 4A).

On the other hand, in phentolamine pretreated animals the average % change in MABP (30.60 ± 1.85 mmHg, antidiuresis (–62.36 ± 1.60 drops / min), and diuresis (36.66 ± 3.87 drops / min) during asphyxia with nicotine (drip) were –2.64 ± 0.29 mmHg (hypotension) ($P<0.001$), -35.39 ± 1.71 drops / min ($P<0.001$) and 28.41 ± 1.89 drops / min ($P<0.001$) respectively (Figs.3B & 4B). The observations indicate that a adrenoceptors are involved in asphyxia-induced hypertension fully and involved partially in antidiuresis and diuresis in both control and nicotinized animals.

4. Effects of asphyxia and asphyxia along with nicotine (drip) induced alterations in blood pressure and urine flow in animals pretreated with prazosin and yohimbine.

Again to see the specific role of a_1 and a_2 adrenoceptors in asphyxia induced alterations of blood pressure and urine flow the control and nicotinized animals were treated with prazosin (0.8 mg/Kg body wt. i.v.) and yohimbine (0.8 mg/Kg body wt. i.v.) respectively. It was observed that asphyxia induced average % change in blood pressure (20.81 ± 1.33 mmHg) was counteracted and the blood pressure was reduced by –6.99 ± 0.29 mmHg ($P<0.001$) and –4.26 ± 0.21 mmHg ($P<0.001$) (Fig.3A) in animals pretreated with prazosin and yohimbine respectively. In prazosin-pretreated asphyxiated animals the average changes in percentage for antidiuresis and diuresis were –20.86 ± 1.04 drops / min and 19.27 ± 2.42 drops / min respectively (Fig. 4A) from that of earlier values of -39.57 ± 1.42 drops / mins and 35.38 ± 2.43 drops / min respectively. The changes were statistically significant ($P<0.001$). On the other hand in yohimbine pretreated animals the asphyxia induced percentage in urine flow for antidiuresis and diuresis were reduced significantly ($P<0.001$) from -39.57 ± 1.42 drops / min to –9.92 ± 0.44 drops / min and from 35.38 ± 2.43 drops / min to 7.74 ± 0.49 drops / min respectively (Fig. 4A).

In asphyxiated animals treatment of prazosin and nicotine (drip) induced an average change in % of MABP was –2.42 ± 0.24 mmHg (control value: 30.60 ± 1.85 mmHg), and that of UF was -28.17 ± 1.57 in case of antidiuresis, (control value: –62.36 ± 1.60 drops / min and 17.09 ± 1.09 drops / min in case of dieresis (control value: 36.66 ± 3.87 drops / min) as shown in Fig. 3B & 4B. The above changes were statistically significant ($P<0.001$). In yohimbine pretreated nicotinized animals, asphyxia caused significant % changes ($P<0.001$) in hypertension and urine flow ,which were –1.94 ± 0.13 mmHg, -7.01 ± 0.58 drops / min (antidiuresis) and 10.04 ± 0.68 drops / min (diuresis) respectively in comparison to the same control value as in case of prazosin (Fig. 3B & 4B). The above observations indicat that in asphyxia and asphyxia with nicotine (drip) induced hypertension, both a_1 and a_2 adrenoceptors are involved fully. In case of urine flows (AD and D) a_1 adrenoceptors are mainly responsible and a_2 adrenoceptors are involved partially.

5. Asphyxia and asphyxia along with nicotine (drip) induced alterations in blood pressure and urine flow in animals pretreated with propranolol.

To find the involvement of the b

adrenoceptors in asphyxia and asphyxia along with nicotine (drip) induced hypertension and urine flow, animals were treated with propranolol at a dose of 0.5 mg/ Kg body wt. (i.v.). In propranolol pretreated animals, the asphyxia induced blood pressure remained unaltered whereas the UF in case of antidiuresis was slightly augmented (from –39.57 ± 1.42 to – 40.31 ± 0.50 drops / min), but in diuresis it remained unaffected (from 35.38 ± 2.43 to 34.14 ± 0.91 drops / min), as shown in Figs. 3 & 4. All the results are insignificant (Fig 3A & 4A).

In propranolol pretreated and nicotinized animal, asphyxia caused no significant alterations in blood pressure and urine flow both in cases of antidiuresis and diuresis (Fig. 3B & 4B). The above findings indicate that in both cases of blood pressure and urine flow (antidiuresis and diuresis)are not mediated through the b adrenoceptors.

DISCUSSION

The experimental study was performed on cat model because cats are more tolerant than rats and rabbits to asphyxia. During asphyxia it was observed that blood pressure was gradually increased and then decreased along with antidiuresis followed by diuresis. Asphyxiation in nicotinized animal caused pronounced hypertension and antidiuresis but there was no significant change in diuresis in comparison to the normal asphyxiated animals (Fig. 1). It was observed that hypertension was totally counteracted in guanethidine sulfate pretreated animal but the antidiuresis and diuresis was partially counteracted in such animals (Fig. 3 & 4). Sympatho-adrenal mechanisms play an important role in cardiovascular responses to asphyxia in mammals. It is well established that asphyxia and nicotine induce release of norepinephrine (NE) from postganglionic sympathetic nerve terminals by activating prejunctional receptor, triggering action potentials in sympathetic nerve terminals. The sympathetic activity in the initial elevation of blood pressure after asphyxia are consistent with a role for the mesenteric system as a key resistance bed that helps to maintain perfusion in other more vulnerable systems (Quaedackers et al., 2004). It is clear from the antagonistic actions of guanethidine sulfate that both asphyxia and nicotine induced release of NE from the sympathetic nerve terminals causes alterations of blood pressure and urine flow (Theobald, 1983; Koley et al., 1987b; Ganog; 2003).

NE acts on both a and b-adrenoceptors and it is known that the a adrenoceptors are more effective to NE than b-adrenoceptors (Ganong, 2003). Otto and their group (1981) reported that the efficacy of epinephrine in adding resumption of spontaneous circulation from asphyxial arrest is due to a-adrenergic receptor stimulation and that b-receptor stimulation is not important in determining outcome. Similar findings were also reported by other investigators (Ghosh and Koley ,1980; Sybertz and Watkins ,1989). To differentiate the roles of a and b-adrenoceptors in such asphyxia along with nicotine induced alterations, phentolamine (a-blocker, Non-selective) and propranolol (b-blocker) were used. In phentolamine pre-treated animals, both asphyxia and

asphyxia along with nicotine-induced hypertension was counteracted completely (Fig. 3). Propranolol has got a little effect on such hypertension (Fig.3). These results indicate that the asphyxia-induced hypertension is mainly mediated through a-adrenoceptors by increasing peripheral vasomotor tone (Kubo and Misu, 1981) in normal and nicotinized animals. Vasopressor response in intact cats during asphyxia is presumably occurring through the release of adrenal medullary hormone, NE (Ghosh and Koley, 1980) and also neurotransmitter from the sympathetic nerve terminals (Kirpekar et al., 1980) causing vasoconstriction.

The asphyxia and asphyxia with nicotine induced antidiuresis was partially counteracted by phentolamine (Fig. 4); but it was slightly augmented, although non-significantly, by propranolol, (Fig. 4). It indicates that antidiuresis is mediated through the a-adrenoceptors. In such animals diuresis is partially mediated through a adrenoceptors (Fig. 4) and also may partially be (non-significantly) influenced by b-adrenoceptors since the diuresis is partially counteracted in phentolamine as well as in propranolol-pretreated animals (not significant) (Fig. 4). So it may be argued that during blocking of b-adrenoceptors by propranolol, the a-adrenoceptors become more dominant and it may create vasoconstriction more profoundly, causing more antidiuresis (Fig. 4).

In Prazosin (α_1, antagonist) pretreated animals, asphyxia induced hypertension was completely counteracted and antidiuresis as well as diuresis are partially counteracted in both control and nicotinized animals (Fig. 3 & 4). These alterations in urine flow are less potent in case of yohimbine ($\alpha_{2,}$ antagonist) pretreated animals than prazosine-pretreated animals (Fig. 3 & 4). It indicates that asphyxia-induced hypertension is mediated through both a_1 and $á_2$ adrenoceptors.

Several studies *in vivo* and *in vitro* have indicated that $\alpha_1$adrenoceptors predominate in renal resistance vessels (Schimitz et al., 1981; Cooper and Malik 1985; Wolff et al., 1987, DiBona and Sawin, 1987) and the α_2-adrenoceptors predominate in the renal tubules (Schmitz et al., 1981; Mc Pherson and Sumers, 1983). De Mey and Vanhoutte (1981) suggested that venous smooth muscle had α_2 adrenoceptors, whereas arterial smooth muscle contained predominately a_1 adrenoceptors. Large number of α_2-adrenoceptors was detected over the renal tubules in the renal cortex, mostly in the proximal tubules (Summers, 1984) and those modulate the reabsorption of Na^+ and water (Schmitz et al., 1981) and only water in the collecting duct (Krothapalli and Suki, 1984). Post functional a-adrenergic receptors in blood vessels of the rat kidney mediating vasoconstriction are predominantly of the α_1 adrenergic receptor subtypes (Langer and Hicks, 1984; Cooper and Malik, 1985).

Stimulation of sympathetic nerves, innervating the rat kidney results in the release of endogenous NE (Schwartz and Eikenburg, 1988) and produces vasoconstriction (Fink and Brody, 1978). Kathryn (1986) stated that renal blood flow is controlled by α_1, not by α_2 adrenoceptors. α_1 adrenoceptors agonist was capable reducing renal blood flow (RBF) to zero and large reduction in RBF could occur with minimum systemic effect. α_1-adrenoceptors are therefore located on renal resistance vessels where

they can presumably modulate renal vascular resistance (Schmitz et al., 1981). NE was apparently exerting its measurable effects predominantly through a_1 adrenoceptors stimulation (Dennis et al., 1987) causing tubular Na^+ reabsorption (DiBona, 1985). Therefore, it may be stated that, NE in response to asphyxia and asphyxia with nicotine, acting on renal a_1-adrenoceptors, produce renal vasoconstriction that causes reduction in RBF, thereby reduction in GFR and consequent reduction in urine formation. On the other hand NE, acting on renal a_2-adrenoceptors increases renal tubular Na^+ and water reabsorption, thereby reduces the volume of urine (Fig. 4). Both these effects by a_1 and a_2 adrenoceptors cause reduction in urine formation, i.e., the manifestation of antidiuresis. So when α_2 adrenoceptors were blocked by yohimbine, asphyxia or asphyxia with nicotine induced NE release failed to act on tubular α_2 adrenoceptors. But under such condition α_1 adrenoceptors mediated renal vasoconstriction was still operative, resulting in reducing of urine volume due to decreased RBF and GFR. Therefore, reduction in urine flow in such animals is due only to the α_1 adrenoceptors mediated renal vasoconstriction and that may be the reason for less but significant antidiuresis in yohimbine pretreated animals (Fig. 4). But when the animals are pretreated with prazosin only, the α_1 adrenoceptors mediated vasoconstriction was withdrawn but α_2 adrenoceptors mediated renal tubular reabsorption was unaffected with asphyxia and asphyxia with nicotine. So antidiuresis was reduced partially but significantly (Fig. 4), yet this reduced antidiuresis was also statistically significant. This indicates that even in absence of α_1 adrenoceptors activity, the renal tubular α_2 adrenoceptors are capable of production of partial but significant antidiuresis.

Therefore asphyxia and asphyxia with nicotine result initial antidiuresis during rising phase of blood pressure which presumably mediated by NE causing activation of both α_1 and α_2 adrenoceptors. During falling phase of blood pressure due to asphyxia and asphyxia with nicotine (drip), there is often diuresis. This diuretic effect is partially mediated by α_1 and α_2 adrenoceptors because both the antagonist do not show significant change in diuresis. Post-asphyxial rise of blood pressure is associated with diuresis (Fig. 1), which is probably due to increase in RBF and obviously increases in GFR. This is because post asphyxial rise in blood pressure as well as urine flow are not altered significantly in yohimbine and prazosin treated or even in guanethidine sulfate pretreated animals (Fig. 3 & 4).

Thus it may be summarized that NE released by asphyxia and nicotine may cause an initial rise in blood pressure along with antidiuresis, which are the effect of α adrenoceptors involvement. Subsequent diuresis during falling phase of blood pressure is not related with á adrenoceptors, though the role of hemodynamics cannot be ruled out. Lastly, it may be stated that the release of NE and involvement of á adrenoceptors are more profound during asphyxia along with nicotine drip in comparison to the normal asphyxia.

REFERENCES

Bartelds B, van Bel F, Teitel DF, Rudolph AM. (1993). Carotid, not aortic, chemoreceptors mediate the fetal cardiovascular response to acute hypoxemia in lambs. *Pediat. Res.* 34: 51–55.

Bellow-Reuss E, Colidres RE, Pastroriza-Muno E, Mueller RA and Gottshalk CW. (1975). Effects of acute unilateral renal denervation in the rat. *J. Clin. Invert.* 56: 208-217.

Blitzer ML, Lee SD and Creager MA. (1996b). Endotheliumderived nitric oxide mediates hypoxic vasodilation of resistance vessels in humans. *Am. J. Physiol.* 271 (3 Part 2): H1182– H1185.

Blitzer ML, Loh E, Roddy MA, Stamler JS and Creager MA. (1996a). Endothelium-derived nitric oxide regulates systemic and pulmonary vascular resistance during acute hypoxia in humans. *J. Am. Coll. Cardiol.* 28 (3): 591–596.

Braaksma MA, Dassel AC and Aarnoudse JG. (1999). Renal responses to prolonged (48 h) hypoxemia without acidemia in the late-gestation ovine fetus. *Am. J. Physiol. Regul. Integ.r Comp. Physiol.* 277: R395–R402.

Brovosky V, Harman M, Dunphy G, Caplea A and Ely D. (1998). CO_2 asphyxia increases plasma norepinephrine in rats via sympathetic nerves. *Am. J. Physiol.* 274(1-2): R19-R22.

Chatkin JM. (2006).The influence of genetics on nicotine dependence and the role of pharmacogenetics in treating the smoking habit. *J. Bras. Pneumol.* 32(6):573-9.

Chen AM, Chen LM, Vaughan A, Sreeraman R, Farwell DG and Luu Q. (2011). Tobacco smoking during radiation therapy for head-and-neck cancer is associated with unfavourable outcome. *Int. J. Radiat. Oncol. Biol. Phys.* 79(2):414-9.

Cock ML, McCrabb GJ, Wlodek ME and Harding R. (1997). Effects of prolonged hypoxemia on fetal renal function and amniotic fluid volume in sheep. *Am. J. Obstet. Gynecol.* 176: 320–326.

Cooper CL and Malic KU. (1985). Prostaglandin synthesis and renal vasoconstriction elicited by adrenergic stimuli are linked to activation of α_1-adrenergic receptors in the isolated rat kidney. *J. Pharmacol. Exp. Ther.* 233: 24-31.

Dennis WW, Frank AG and Jack WS. (1987). *In vivo* assessment of rat renal a-adrenoceptors. *The J. of Pharmacol and Expt. Ther.* 241: 472-476.

De Mey J and Vanhoutte PM. (1981). Uneven distribution of post junctional α_1 and á$_2$ like adrenoceptors in canine arterial and venous smooth muscle. *Circ. Res.* 48: 875-884.

DiBona GF. (1985). Neural regulation of renal tubular Na^+ reabsorption and rennin secretion. *Fed. Proc.* 44: 12816-12822.

DiBona GF and Sawin LL. (1987). Role of renal α_1-adrenergic receptors in spontaneously hypertensive rats. *Hypertension.* 9: 41-48.

Downey HF. (1990). Adrenal release of catecholamine in coronary and myocardial response to nicotine. *Adv. Exp. Med. Biol.* 273: 327 – 337.

Fink GD and Brody MJ. (1978). Continuous measurement of renal blood flow changes to renal nerve stimulation and intra-arterial drug administration in the rat. *Am. J. Physiol.* 3: H219-H222.

Ganong WF, (2003). Dynamics of blood lymph flow. *Review of Medical Physiology.* 21st edition, Appleton and Lange, Stamford CT, Simon A and Schuster Company, USA, pp. 550-567.

Gebber GL. (1969). Neurogenic basis for the rise of blood pressure evoked by nicotine in the cat. *J. Pharmacol. Exp. Ther.*166: 255-263.

Ghosh S and Koley BN. (1977). Vasomotor imbalance during asphyxia in intact cats. *Ind. J. Physiol. & Allied Sci.* 31: 106.

Ghosh S and Koley BN. (1980). Role of adrenergic receptors on asphyxic vasopressor response. *J. Expt. Biol.* 18: 1-2.

Giussani DA, Spencer JA, Moore PJ, Bennet L and Hanson MA. (1993). Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal

sheep. *J Physiol,* 461: 431–449.

Green LR, Bennet L, Robson S and Hanson MA. (1997). The role of carotid chemoreceptors in the effects of hypoxia on renal blood flow in the late gestation sheep fetus. *Exp Physio.* 82: 183–192.

Haldar R, Haque MZ, Koley J and Koley BN. (2001). Neurotransmitters involved in nicotine induced alteration in urine flow. *Ind. J. Physiol. & Allied Sci.* 55(1): 1-6.

Kathryn HM, Lisa M, Steven G and Theodore AC, (1986). α_2 adrenergic receptors localization in the rat heart and kidney using autoradiography and tritiated Rauwolscine. *The J. of Pharmacol and Expt. Ther.* 236: 542-547.

Kirpekar SM, Garcia AG and Prat JC. (1980) Action of nicotine on sympathetic nerve terminals. *J. Pharmacol. Exp. Ther.* 213: 133-138.

Koley BN and Mukherjee SR. (1964). Studies on asphyxia and vasomotor imbalance. *Ind. J. Physiol. & Allied Sci.* 18(3&4): 119-128.

Koley J, Das M, Basak AK and Koley BN. (2001). Ventricular nociception induced vesicular motility and urine flow: Their relationship. *Ind. J. Pharmacol.* 45: 463-469.

Koley J, Saha JK and Koley B. (1987b). Phermacological and electrophysiological analysis of the effect nicotine on cat blood pressure. *Arch. Int. Phermadyn. Ther.* 287(1): 31-47.

Krothapalli RK and Suki WN. (1984). Functional characterization of the á-adrenergic receptors modulating the hydroosmotic effect of vasopressin on the rabbit cortical collecting tubule. *J. Clin. Invest.* 73: 740-749.

Kubo T and Misu Y. (1981). Increased pressor responses to nicotine in spontaneously hypertensive rats, *Jpn. J. Pharmacol.* 31(2): 221-226.

Langer SZ and Hicks PE. (1984). á-adrenoceptor subtypes in blood vessels. Physiology and Pharmacology. *J. Cardiovasc. Pharmacol.* 6: S547-S558.

Marano G, Ramirez A, Mori I and Ferrari AU. (1999). Sympathectomy inhibits the vasoactive effects of nicotine in conscious rats. *Cardiovasc. Res.* 42(1): 201-205.

Mayhan WG. (1999). Acute infusion of nocotine potentiates NE induced vasoconstriction in the hamster cheek pouch. *J. Lab. Clin. Med.* 133(1): 48-54.

Meister B, Dagerlind A, Nicholase AP and Hokfelt T. (1994). Pattern of messenger RNA expression for adrenergic receptor subtypes in rat kidney. *J. Pharmacol. Exp. Ther.* 268: 1605-1611.

McPharson GA and Summers RJ. (1983). Evidence from binding studies for α_2-adrenoceptors directly associated with glomeruli from rat kidney. *Eur. J. Pharmacol.* 90: 333-341.

Odoni G, Ogata H, Viedt C, Amann K, Ritz E and Orth RS. (2002). Cigarette smoke condensate aggravates renal injury in the renal ablation model. *Kidney international.* 61: 2090-2098.

Otto CW, Yakaitis RW and Blitt CD. (1981). Mechanism of action of epinephrine in resuscitation from asphyxial arrest. *Crit. Care. Med.* 9(4): 321-24.

Pinto FR, Matos LL, Gumz Segundo W, Vanni CM, Rosa DS, Kanda JL. (2011). Tobacco and alcohol use after head and neck cancer treatment: influence of the type of oncological treatment employed. *Rev. Assoc. Med. Bras.* 57(2):171-76.

Quaedackers JS, Vincent R, Erik H, Alistair JG and Laura B. (2004). The role of the sympathetic nervous system in post-asphyxial intestinal hypoperfusion in the preterm sheep fetus. *The Journal of Physiology*. 557.3: 1033–1044

Ritz E, Benck U, Frank E, Keller C, Seyfarth M, and Clorius J. (1998). Effect of smoking on renal hemodynamic in healthy volunteers and in patients with glomerular disease. *J Am. Soc. Nephrol.* 9(10): 1798-1804.

Robillard, JE, Weitzman, RE, Burmeister, L and Smith, FG Jr., (1981). Developmental aspects of the renal response to hypoxemia in the lamb fetus. *Circ Res.* 48: 128–138.

Schmitz JM, Grahan RM, Sagalowsky A and

Pettinger WA. (1981). Renal α_1 and α_2 adrenergic receptors: Biochemical and pharmacological corrections. *J. Pharmacol.xp. Ther.* 219: 400-406.

Schwartz DD and Eikenburg DC. (1988). Enhanced endogenous neurotransmitter overflow in the isolated perfused rat kidney after chronic epinephrine administration: Lack of a prejunctional α-adrenoceptors influence. *J. Pharmacol. Exp. Ther.* 244: 11-18.

Summers RJ. (1984). Renal a receptors. *Fed. Proc.* 43: 2917-2922.

Sybertz EJ Jr. and Watkins RW. (1989). Preclinical pharmacologic properties of dilevalol, an antihypertensive agent possessing selective α_2 agonist mediated vasodilation and α antagonism. *Am. J. cardiol.* 63 (19): 31-61.

Theobald RJ Jr. (1983). Evidence against purinergic nerves of the cat. *J. Auton. Pharmacol.* 3: 235-239.

Toll BA, Brandon TH, Gritz ER, Warren GW and Herbst RS. (2013). AACR Subcommittee on Tobacco and Cancer. Assessing tobacco use by cancer patients and facilitating cessation: an American Association for Cancer Research policy statement. *Clin Cancer Res.* 19(8):1941-8.

Volpe, J., (2001). Neurology of the newborn. Saunders, Philadelphia, 217- 394.

Wassink G, Bennet L, Booth LC, Jensen EC, Wibbens B, Dean JM and Gunn AJ. (2007). The ontogeny of hemodynamic responses to prolonged umbilical cord occlusion in fetal sheep. *J Appl Physiol.* 103: 1311–1317.

Wolff DW, Gesek FA and Strandhoy JW. (1987). In vivo assessment of rat renal α-adrenoceptors. *J. Pharmacol. Exp. Ther.* 241: 472-476.

World Health Organization (2011). Geneva: WHO report on the global tobacco epidemic: warning about the dangers of tobacco?

Zapata P, Zuazo A and Leados F. (1976a). Acute changes in ventilation and blood pressure induced by inhalation of tobacco smoke. *Arch. Int. Pharmacodyn.* 219:116-127.