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# Asphyxia and Nicotine Induced Alterations in Blood Pressure and Urine Flow: Role of Spinal Center

**Sandip Kumar Sinha***

**Abstract**

*Background:* Asphyxia is the condition of increased $P_{CO2}$ and decreased $P_{O2}$ in blood. It is of general opinion that carbondioxide excess and oxygen lack stimulate the hypothalamus and as a result activate the sympathetic system causing sympatho-adrenal discharge has described that the activation of the sympathy-oadrenal system depends on an intact brain stem and spinal cord, although there is probably some direct action of $CO_2$ on the adrenal medulla. Thus, the effect of asphyxia on the hypothalamus and medullary vasomotor centre directly or indirectly through chemo-receptors is the increased systemic pressor response and renal functions due to increased sympatho-adrenal activity. *Aims and Objective:* In the present study, try to find out the role of spinal centre in asphyxia and asphyxia with nicotine (drip) condition at the time of hypertension and urine flow. *Methods:* Experimental asphyxia (40 to 90 seconds) was done after tracheostomy and nicotine drip (8–10 drop/min) used intravenously through femoral vein. The spinal cord was opened by laminectomy at the level of $C_7$–$C_8$. Both ends of the opened spinal cord was tied by cotton thread and then sectioned when needed. *Results:* In such spinal animals asphyxia failed to alter the blood pressures both in control and nicotinised animals. But the changes in urine flow in such animals; the diuresis is partially counteracted whereas the antidiuresis is slightly but significantly augmented in comparison to the control. *Conclusion:* Therefore, from the above observations of the present study, it may be argued that asphyxia and asphyxia with nicotine (drip) act through supraspinal centers for the alterations in blood pressure and urine flow.

**Keywords:** Asphyxia; Nicotine; Hypertension; Urine Flow; Supra Spinal Centers.

## Introduction

Sherrington (1909) discussed the rise of arterial blood pressure in decapitated cats following asphyxiation of the animals. He explained that the rise of arterial blood pressure was due to lack of oxygen. After him Kaya and Starling (1909) also worked on that problem and showed conclusively that there was a marked difference between the behaviour of the spinal cord and medulla (Supraspinal centre) with respect to two blood gases, $O_2$ and $CO_2$. They postulated that increased $CO_2$ tension excited markedly the medullary centres whereas increased $CO_2$ and diminished $O_2$ tension used to excite lower centers. Spinal centre are responsive to gaseous content of blood (Mathisons 1910, Catheart and Clark, 1915).Guyenet (2006) reported that dysfunctional reflexes and/or increased activity of the para-ventricular nucleus of the hypothalamusrostral ventrolateral medulla (PVH–RVLM) axis are factors that are currently suspected of contributing to the chronic elevation of barosensitive sympathetic efferents in many forms of hypertension. It was also reported that perinatal asphyxia generates a global transient hypoxia-ischemia status that damages the brain, spinal cord (Dorfman et al., 2009; Loidl et al., 1994). Kaya and Sterling (1909) pointed out that the spinal centers were sensitive to $CO_2$ only when there was also $O_2$ lack. A fall in blood pressure was also observed by Koley and Mukherjee (1964) during asphyxia in spinal cat ($C_7$–$T_1$).

The cardiovascular responses induced by nicotine, injected or inhaled through tobacco smoke,

**Author's Affiliations:** *Assistant Professor, Department of Human Physiology with Community Health, Vidyasagar University, Paschim Medinipur,Pin: 721102, West Bengal, India.

**Corresponding Author: Sandip Kumar Sinha,** Assistant Professor, Department of Human Physiology with Community Health, Vidyasagar University, Paschim Medinipur,Pin: 721102, West Bengal, India.

Email: sinha_sandip@mail.vidyasagar.ac.in, sandip_sinha3@yahoo.co.in

are of diverse nature and complex. The moderate rise of blood pressure by high doses of nicotine (50 mg / kg) in spinal cats ($C_2$) has been described to be due to the release of catecholamines from adrenal glands (Armitage, 1965). The results regarding the role of central vasomotor centers in producing blood pressure changes by nicotine are described in different ways by several investigators (Armitage and Hall, 1967, 1969; Ginzel 1967; Gebber 1969). A direct action of nicotine on central vasomotor areas is supposed to be the primary factor involved in the pressor response evoked by nicotine (Armitage and Hall, 1969). According to Kubo and Misu (1981), the pressure effect of nicotine may results from the activation of central nicotinic receptor sites, which may cause the release of catecholamines both from the adrenal medulla and adrenergic nerve terminals.

It is also well known that central nervous system plays a crucial role in control of sympathetic and parasympathetic nervous system. Role of sympathetic and parasympathetic system has been discussed earlier by the present author role in asphyxia and nicotine induced blood pressure and urine flow alterations. It has been shown already that sympatho-adrenergic system has got definite role in such alterations. The parasympathetic nervous system has also got some role in such alterations. When the release of NE from the sympathetic nerve terminal was blocked by guanethidine sulfate, asphyxia and asphyxia with nicotine (drip) both failed to generate any alterations in cardio-renal functions. This sympatho-adrenomedullay discharge originating from the spinal cord neurons may be the probable cause of such asphyxia and asphyxia with nicotine induced vasopressor response in intact cats. These observations led the present author to investigate into the problem further by studying the spinal cord vasomotor neurons on such asphyxia and asphyxia with nicotinic induced vasopressor response in spinal cats, transecting the cord at $C_7$-$C_8$ level.

## Methods and Materials

### *Experimental design*

Experiments were carried out in 16 normal adult cats of either sex, weighing about 2 to 3 kg. All the animals were classified into two groups consisting of eight animals in each group. Group- I represented the asphyxiated animals without nicotine drip. Group-II represented asphyxiated animals along with nicotine drip. To observe the role of spinal vasomotor centre, spinal transection was performed at the cervical ($C_7$–$C_8$) level. In these two groups of animal spinal section were applied.

### *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 anaesthetic 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).

### *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.

*Preparation of Spinal animals*

The spinal cord was opened by laminectomy at the level of $C_7$–$C_8$. Both ends of the opened spinal cord was tied by cotton thread and then sectioned when needed. Before transection 2% lignocaine was injected in the spinal cord at the $C_7$–$C_8$ level to avoid spinal shock following the method of Koley and Mukherjee, 1964; Koley et al., 1984b. To revive from spinal shock the animal was left for half an hour after spinal transection.

*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).

*Administration of Drugs*

All the drugs were dissolved or diluted in the normal saline solution (0.9gm% $NaCl_2$) freshly before the experiments. Desired quantities of tested drugs were introduced through the three-way stopcock attached with the femoral vein catheter in all 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.) also used intravenously through the right femoral vein in a dose ranging from 20-60 çgm / Kg., since in cats on administration of 10–20 ìgm / Kg body weight / min. intravenously, plasma level of nicotine as measured (Gebber 1969; Zapata et al 1976a) is about 40–70 çgm / ml which was approximately similar to smoker's nicotine level in blood.

*Materials*

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)

Calcium chloride dehydrate (E. Merck limited, India) Heparin (Biological E Ltd. India)

Lignocaine Hydrochloride ('Gesicain' 2%, Suhrid-Geigy, India)

## Results

*Effects of asphyxia on blood pressure and urine flow in both 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 is 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 comparatively increase rapid in blood pressure. Blood pressure returned back to initial level slowly over a period of 5–10 minutes and simultaneously urine flow also returned to its initial level (Fig. 1).

During asphyxia average resting MABP increased 25.64 % (from 85.85 ± 1.93 to 107± 2.54 mmHg, $P < 0.001$) and changes in 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, $P < 0.05$) (Fig. 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 & 4).

After the intravenous application of nicotine drip (10–20 µgm / kg / min) blood pressure began to rise slowly and stabilized at a constant level after 20–30 min. When the pressure began to rise, urine flow also decreases considerably (Fig.1). Due to asphyxia (40-90 seconds) resting pressure was further increased 28.84 % (from 104 ±2.37 to 134 ±2.67, *P <0.001*) along with more 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 observed, 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 / min, *P <0.001*) (Fig. 1B & 2). All these cardio-renal changes are 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) is 30.60 ± 1.85 mmHg and antidiuresis and diuresis was –62.36 ± 1.6 and 36.66 ± 3.87 drops / min respectively (Fig.3 & 4). Thus it is clear that in nicotinized condition asphyxic effects on hypertension and urine flow are more profound and intensive (Fig.1B). However there was no significant % change in diuresis in nicotinized state in comparison with the results of asphyxia without nicotine.

*Effect of asphyxia on blood pressure and urine flow in spinal ($C_7$–$C_8$) animals.*

Before the performance of spinalectomy at $C_7$ -$C_8$, the asphyxia induced average % change in mean arterial blood pressure was 20.81 ± 1.33 and in urine flow during antidiuresis was –39.57 ± 1.42 and diuresis was 35.38 ± 2.43 (Fig.3 & 4). In spinal animals ($C_7$–$C_8$), asphyxia induced alterations in hypertension was completely counteracted but at the same time the antidiuresis become slightly augmented and diuresis was partially counteracted. So in such spinal animals the average % change in mean arterial blood pressure and urine flow during asphyxia alone were –6.03 ± 0.47 (P<0.001), -49.10 ± 1.60 (P<0.001) (antidiuresis) and 20.25± 2.11 (P<0.001) (diuresis) respectively (Fig.3 & 4). It is apparent that asphyxia induced hypertension is completely and diuresis is partially mediated through the higher centers i.e. the supraspinal ($C_7$–$C_8$) level.

*Effect of asphyxia along with nicotine (drip) on blood pressure and urine flow in spinal ($C_7$–$C_8$) animals.*

In case of control nicotinised animals, before spinal transection the asphyxia induced average % change in mean arterial blood pressure, antidiuresis and diuresis were 30.60 ± 1.85, -62.36 ± 1.60 and 36.66 ± 3.87 respectively (Fig.3 & 4). In such animals similar results were observed as saw in control asphyxiated spinal animals. In such nicotinised animal asphyxia induced average % change in hypertension was –4.06 ± 0.74 (P<0.001) (Fig.3), urine flow during antidiuresis was –68.64 ± 2.45 (P<0.02) and during diuresis was 25.39 ± 1.10 (P<0.001) (Fig.4), indicating that asphyxia induced hypertension is completely and diuresis is partially mediated through the spinal vasomotor centres.

**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 pattern. 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.

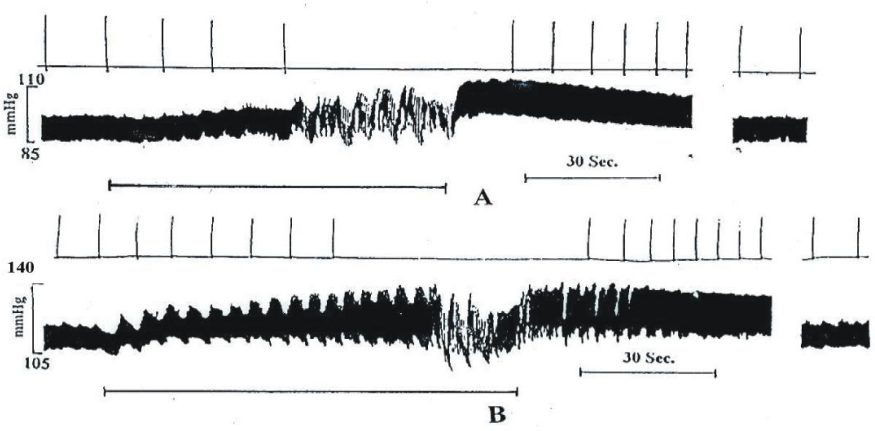


**Fig. 2:** Effects of asphyxia and asphyxia along with nicotine drip on cardio-renal changes. Bar graphs showing the *average mean arterial blood pressure (mmHg)*during control (n= 36) and experiment (n= 37) **(A)** *Upper panel* and *urine flow (drops/min)*as initial (n=28), antidiuresis (n=29) and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

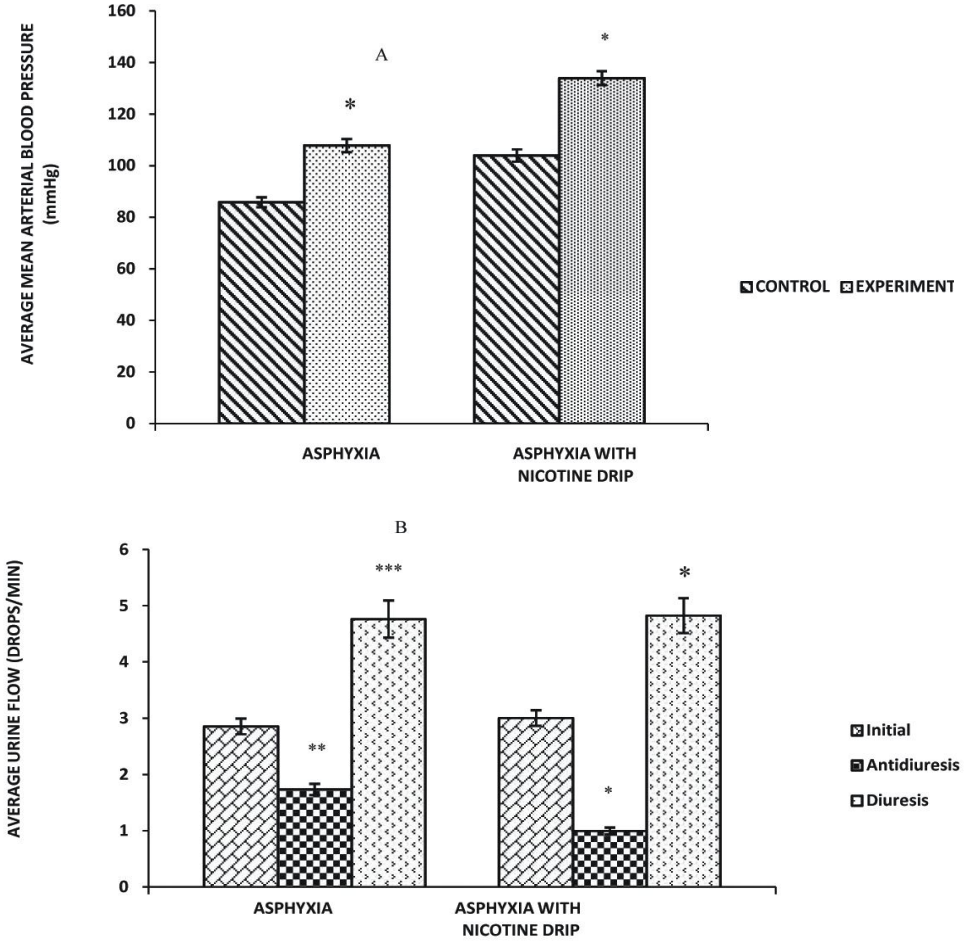



**Fig.3.** Effects of spinalectomy on hypertension. Bar diagram illustrating the average % change in mean arterial blood pressure (mmHg) during *asphyxia* and *asphyxia with nicotine drip* in spinalectomy conditions. Number with in the parenthesis indicates the number of observations (n).Valuesare means ± SEM, *$P$ < 0.001compared with control.

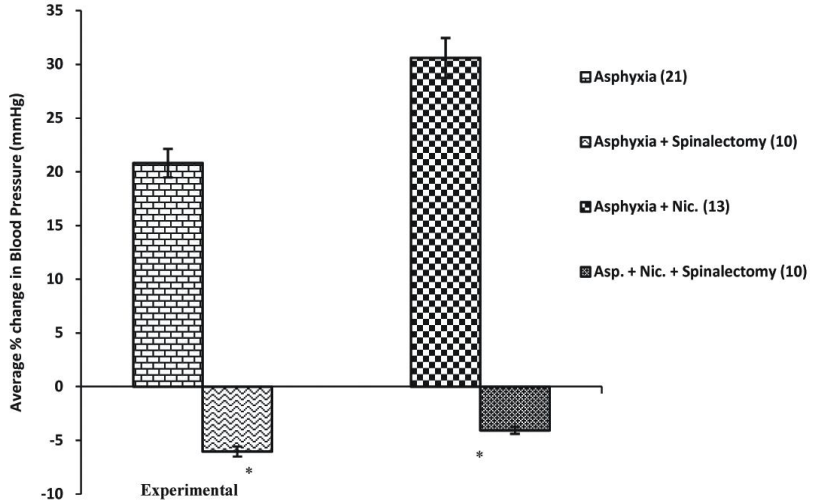
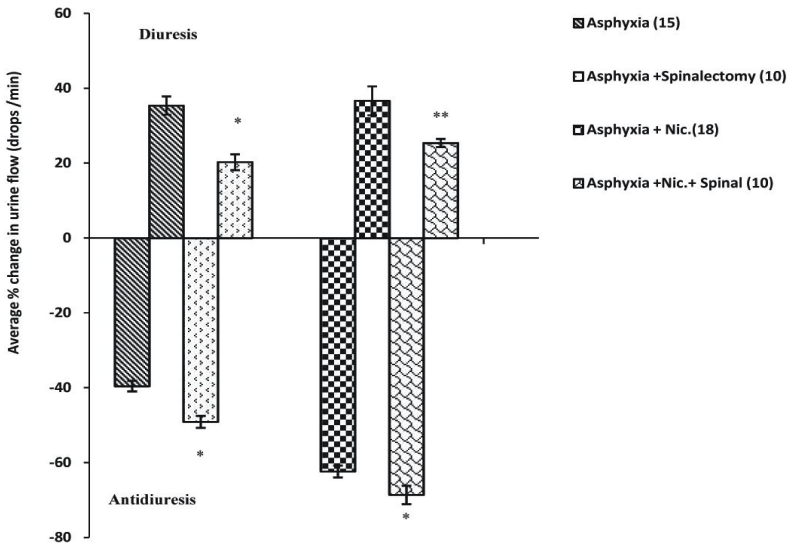


**Fig.4:** Effects of spinalectomy on renal functions like urine flow. Bar graphs illustrating the average % change in *urine flow (drops /min)* during *asphyxia* and *asphyxia with nicotine drip*in spinalectomy conditions. Number with in the parenthesis indicates the number of observations (n).Valuesare means ± SEM. *$P$ < 0.001and**$P$ < 0.02 compared with control.




## Discussion

In various study, it has suggested that the sympathetic and sympatho-adrenal discharges, to be the cause of asphyxic pressor response in intact animals. It has also been discussed that the final pathway of pressor response in animals following asphyxia is through the activation of sympathetic, preferably with the adreno-medullary and sympathetic nerve terminal discharge.

In this investigation, the involvement of supraspinal centers has been studied in asphyxia and asphyxia with nicotine (drip) induced alterations in blood pressure and urine flow. In this case spinal animals were prepared by transecting the spinal cord at the level of $C_7$-$C_8$. In such spinal animals, the parasympathetic (of sacral origin) and sympathetic efferent of spinal cord have been isolated from the supraspinal centre is from the control of higher centre. In such spinal animals asphyxia failed to alter the blood pressures both in control and nicotinised animals (Fig.3). But the changes in urine flow in such animals; the diuresis is partially counteracted whereas the antidiuresis is slightly but significantly augmented in comparison to the control (Fig. 4).

The above observations indicate that during asphyxia autonomic centers are stimulated the peripheral sympathetic outflow, which originated from the spinal cord $T_1$–$T_{12}$, is not excited because of transection at the level of $C_7C_8$. Thus the sympatho-adrenal discharge, which is initiated through activation of splanchnic divisions of sympathetic fibers, is not possible due to absence of usual neuronal connections from the higher centers.

Cardio-vascular homeostasis in normal individual is maintained by the peripheral autonomic outflow and is finally controlled by the integrated functions of higher centers like cerebral cortex, reticular formations, hypothalamus, medulla and spinal cord. Spinal cord vasomotor centers along with the supraspinal vasomotor centers control the excitatory tone of peripheral arterioles. Vasoconstrictor fibers leave the spinal cord between the first thoracic and second lumber segments; transection below the level of the second lumber produce little fall of blood pressure while section at the first thoracic level causes a fall of blood pressure equal to that resulting from destruction of the vasomotor centre (Keele and Neil, 1971). Armitage (1965) has reported that high dose of nicotine (50 mg / kg) in spinal cats ($C_2$) may result in moderate rise of blood pressure. A direct action of nicotine on central vasomotor areas is supposed to be the primary factor involved in the pressure response evoked by nicotine (Armitage and Hall, 1969). Further studies demonstrate that the pressure response of nicotine may result from the activation of central activation of central nicotinic receptor sites that may results in the release of catecholamines both

from the adrenal medulla and sympathetic adrenergic nerve terminals (Kubo and Misu, 1981). The observations of the present study corroborates with the previous observations of Armitage and Hall (1969) and Kubo and Misu (1981).

It has been described earlier that asphyxia is the condition of increased $P_{CO2}$ and decreased $P_{O2}$ in blood. It is of general opinion that carbondioxide excess and oxygen lack stimulate the hypothalamus and as a result activate the sympathetic system causing sympathoadrenal discharge (Tenney and Lamb, 1965). Tenney (1956a, 1956b) has described that the activation of the sympathoadrenal system depends on an intact brain stem and spinal cord, although there is probably some direct action of $CO_2$ on the adrenal medulla. Thus, the effect of asphyxia on the hypothalamus and medullary vasomotor centre directly or indirectly through chemo-receptors is the increased systemic pressor response due to increased sympatho-adrenal activity. The sympathoadrenal response is of fundamental significance of homoeostasis during $CO_2$ stress (Tenney and Lamb, 1965).

In the present study in spinal ($C_7$–$C_8$) animals, asphyxia and nicotine induced diuresis are partially counteracted whereas antidiuresis is slightly but significantly augmented. Therefore, it may be argued that, these alterations are due to lack of medullary control over the spinal cord vasomotor centre, which changes the renal hemodynamics. As the blood pressure is depressed in such spinal animal which in turn causes less renal blood flow, resulting reduction of glomerular filtration rate, thereby increase of antidiuresis in such spinal ($C_7$–$C_8$) animals. As the renal blood flow becomes reduced during asphyxia the diuresis also partially reduced after the release of clamping, in such spinal ($C_7$–$C_8$) animal.

It may therefore, be suggested that in intact animals, having normal neural connections in between the higher vasomotor centre and the spinal cord sympatho-adrenal system, asphyxia and asphyxia with nicotine drip induced effect on systemic blood pressure and urine flow is pre-dominantly vasopressor due to excitation of the sympatho-adrenal system by the higher vasomotor centre. In such animal $CO_2$ may cause vasodilatation by acting directly on the vascular smooth muscle but remains overshadowed by the predominating vasoconstrictor response of sympatho-adrenal discharge triggered by the stimulation of higher vasomotor centers. So, in spinal animals, having spinal cord transected at the level of $C_7$-$C_8$ thereby having lack of normal neural connections, in between the spinal cord vasomotor centre ($T_1$–$L_2$) along with sympatho-adrenal system and the higher vasomotor centre, the asphyxia and asphyxia with nicotine induced response is predominantly depressor, only because of local action of $CO_2$ on the smooth muscle of peripheral vascular beds (Fleishman et al., 1957; Diji, 1959; Folkow; 1960). Thus fall in blood pressure and change in urine flow in spinal animal ($C_7$–$C_8$) following asphyxia and asphyxia with nicotine (Fig.3 & 4) may be due to the decrease of peripheral resistance and failure of higher vasomotor centre to excite the sympatho-adrenal system in absence of usual neural connections in between them. Thus, when the sympatho-adrenal system is minimized the ability of the animal to survive asphyxia and nicotinic stress is limited, suggesting the beneficial effect of catecholamines release in counteracting asphyxia and asphyxia with nicotine induced vasomotor imbalance. Therefore, form the above observations of the present study, it may be argued that asphyxia and asphyxia with nicotine (drip) act through supraspinal centers for the alterations in blood pressure and urine flow.

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