CHAPTER 2

METHODS AND MATERIALS
2.1. Experimental animal

Experiments were carried out on 143 adult cats of either sex weighing 2-3 kg. The animals were kept under overnight fasting with water ad libitum before the experiments.

2.2. Animal Preparation

2.2.1. Anaesthesia:

Anaesthesia was initially induced with anaesthetic ether. Then α-chloralose solution in normal physiological saline (0.9%) was administered intravenously at a dose of 60 mg/kg body weight. The depth of anaesthesia was assessed by the presence of pupillary reflex, auditory reflex etc. of the animal. The depth of anaesthesia was maintained by giving α-chloralose at a dose of 10 mg/kg body weight as and when required.

2.2.2. Cannulation of the femoral vein:

A portion of the femoral vein (FV) was cleared from the surrounding connective tissues and tied at the rear end to prevent bleeding. A small incision was given above the tie and the normal saline (0.9%) filled polyethylene catheter was introduced through the small hole. The catheter was fixed with the vein with another thread. The other end of cannula was connected with the drip bottle via a three-way stop cock (Fig.2.1A).

2.2.3. Cannulation of the trachea:

A low tracheostomy was performed at the C₅-C₇ level and a “T” shaped glass cannula was inserted into the trachea. The cannula was tied with the trachea to keep it in position. Artificial respiration was provided when required with the help of a Starling Ideal Respiratory Pump (INCO, India). The depth of respiration was controlled by the stop cock attached with the outlet tube of the tracheal cannula (Fig.2.1A).

2.2.4. Cannulation of the femoral artery and recording of blood pressure:

The femoral artery (FA) was isolated and cleared from the surrounding tissues. Two threads were passed beneath the artery. The lower thread was tied and a small incision was made on the arterial wall holding the upper thread in such a manner so that blood could not come out
through the hole. A polyethylene catheter filled with heparinised normal saline was inserted through the hole into the abdominal aorta upto the level of the renal artery and tied with the arterial wall to keep it in position. Heparinised saline was administered intraarterially to prevent blood clotting (Fig.2.1A).

The abdominal arterial blood pressure was recorded on an INCO polygraph (Model 201, Recorders and Medicare System, Chandigarh, India) with the help of an INCO pressure transducer (Model T-301). The transducer was filled with heparinised saline solution and attached with the arterial cannula with the help of a three-way stop cock. The blood pressure recorded from the artery at the level of renal artery gives the estimate of renal perfusion pressure.

Mean blood pressure was calculated according to the formula:

\[ \text{Mean Blood Pressure (BP)} = \text{Diastolic Pressure (DP)} + \frac{1}{3} \text{Pulse Pressure (PP)}, \]

where, \( \text{Pulse Pressure (PP)} = \text{Systolic Pressure (SP)} - \text{Diastolic Pressure (DP)}. \)

2.2.5. Cannulation of the urinary bladder and recording of the bladder movement:

A midline incision was given at the lower abdomen and through this opening the urethra was traced out and cleared from the surrounding tissues. Two threads were passed beneath the urethra. With one of the threads the lower portion of the urethra was tied. An incision was given on the urethral wall to make a small hole through which a polyethylene cannula of approximately 2 mm diameter was inserted up to the middle of the urinary bladder (UB). The cannula was tied by the other thread with the urethra to keep it in position. The opening was sutured carefully after completion of the surgical procedure. The bladder was first emptied and then 10-15 ml warm (37°C) normal saline solution was introduced into the bladder. The volume of the bladder was checked time to time and maintained with a constant volume during the period of the experiment. An INCO pressure transducer was filled with normal saline solution and attached with a three-way stopcock. The transducer was connected to the INCO polygraph for recording the movement of the bladder in terms of intravesicular pressure (IVP) (Fig.2.1A). Before recording the vesicular motility, the animals were paralysed with intravenous pancuronium bromide (1 mg/kg) injection to eliminate the influence of skeletal muscles.
Fig. 2.1. Schematic diagram of the different preparations of the experimental animal.
2.2.6. Recording of urine flow:

A small lateral incision was given on either side at the L4-L5 region. The ureter (UR) was cleared from the surrounding connective tissues and tied with a fine thread to prevent the backflow of urine. Above the knot, a fine incision was given on the ureter wall very carefully under the stereoscopic dissecting microscope (Vickers' Instrument, U.K.). A fine polyethylene catheter was inserted through the hole up to the renal pelvis. The catheter was kept in position by tying it with the ureter wall. The free end of the catheter was connected to a drop recorder coupled to an INCO polygraph and urine drops were recorded as one spike per drop (Fig. 2.1A). Spikes per minute were counted to calculate the urine flow as drops/min.

2.2.7. Maintenance of body temperature, fluid volume and pH:

Throughout the period of experiment, the rectal temperature of the animal was monitored continuously and it was maintained at 37±1°C with the help of a heating pad.

5% glucose in physiological saline solution was administered through drip feed via the femoral vein at a rate of 0.12 ml/kg b.w./min. throughout the tenure of the experiment to maintain fluid volume.

pH of the blood was checked time to time during the experiment and it was maintained by infusion of 1M NaHCO₃ in glucose-saline solution or by controlling the artificial ventilation.

2.2.8. Opening of the chest:

Chest was opened keeping the animal under artificial ventilation. A midline incision was given at the ventral surface of the thorax. After removing the skin and muscle layers, 2nd-5th left thoracic ribs were removed carefully so that no damage was caused to lung or heart. After completion of the surgical procedure the cavity was covered with a cotton film soaked with normal saline.

2.2.8.1. Exposing the heart:

A longitudinal incision was given on the pericardium to expose the ventricle very carefully.
Four corners of the cut pericardium was tied with fine cotton threads and a pericardial cradle was made. This pericardial cradle helped in applying drugs locally and washing the ventricular surface after drug application without spreading the drugs over the neighbouring organs.

2.2.8.2. Induction of ventricular ischaemia :

The main branch of the left anterior descending coronary artery (LAD) was cleared carefully from the connective tissues under the stereoscopic dissecting microscope (Vicker’s Instruments, UK) carefully. A fine thread was passed under the artery with the help of a fine curved suture needle. The free ends of the thread were passed through a small polyethylene tube to make a snare (SN) (Fig.2.1B). LAD was occluded temporarily by pulling the snare when required.

2.2.9. Denervation :

In some sets of experiments, denervation was done with vagi (VAG), inferior cardiac nerve (ICN), thoracic sympathetic chains (TSC) (Fig.2.1C), hypogastric nerve (HGN) (Fig.2.1D) and renal sympathetic nerve.

2.2.9.1. Vagus :

Vagus nerves were sectioned either at cervical or thoracic level. The nerves were isolated from the sympathetic trunk and two knots were placed at 5 mm apart. The nerves were sectioned in between the knots after placing small ice cube over the nerves to prevent shock (Fig.2.1C).

2.2.9.2. Inferior Cardiac Nerve :

Inferior cardiac nerves (ICNs) were isolated under the stereoscopic dissecting microscope as they descend from the left stellate ganglion. Two knots were placed at 5 mm apart and the nerve was sectioned in between the knots after cooling with ice cube (Fig.2.1C).

2.2.9.3. Stellate ganglia :

The stellate ganglion (ST) of either side was isolated and cleared from the surrounding tissues. All the nerve connections of the ganglion were sectioned and the ganglion was removed (Fig.2.1C).
2.2.9.4. Thoracic sympathetic chain :

The thoracic sympathetic chain (TSC) of either side was traced out and cleared from the surrounding tissues. The chain was sectioned below the stellate ganglion (ST). The T1-T6 sympathetic rami were also sectioned (Fig. 2.1C).

2.2.9.5. Hypogastric Nerve :

The hypogastric nerves (HGN) were exposed retroperitoneally by giving a lateral incision at the L1-L4 level. The abdominal viscera were pushed aside and the nerves along with the ganglia were cleared from the surrounding tissues. The nerves were cooled with ice cubes and sectioned below the inferior mesenteric ganglia (IMG) or the inferior mesenteric ganglia was removed (Fig. 2.1D).

2.2.9.6. Renal sympathetic :

The renal sympathetic nerves were identified and isolated under the stereoscopic dissecting microscope along the wall of the renal artery. It was cleared from the surrounding tissues and sectioned.

2.2.10. Adrenalectomy :

Adrenalectomy was performed as described by Armitage (1965). The adrenal veins and arteries were isolated carefully with the aid of a stereoscopic dissecting microscope. Double ties were placed around the adrenal veins and arteries and the glands were removed. The animals were allowed to rest for about 60 mins after the surgical procedure.

2.2.11. Carotid occlusion :

The carotid arteries of either side were isolated from the vagosympathetic trunk and other surrounding tissues. Two threads were put around each artery above and below the carotid sinus. Carotid occlusion was done by pulling and clamping the threads to prevent blood flow through the arteries.

2.2.12. Spinal transection :

Laminectomy was performed at the leve of C7-C8 placing the cat dorsally. 0.1 ml of 2%
Lignocaine was injected into the dura mater of the spinal cord to prevent the spinal shock. After 5 minutes, two knots were placed at 1 cm (approximately) apart on the spinal cord. Then the cord was transected and covered with cotton soaked with cold saline and the skin was sutured carefully. The blood pressure, respiration and body temperature were carefully monitored. After at least 2 hours of spinal transection experiments were performed on these spinal cats.

2.2.13. Decerebration:

Decerebration was performed at the midcollicular level under ether anaesthesia following the method of Sherrington (1898); Downmann and Hussain (1958). The cat was placed dorsally. The skin and muscle layers of the left side of the head were removed. A hole was made on the left lateral wall of the cerebral fossa with the help of a dental burr. A scalpel was introduced through this hole and transection of the brain stem was performed. Completion of decerebration was confirmed by checking the rigidity of the limbs, absence of pupillary reflex etc. After 2 hours of decerebration, experiments were performed in such cats.

2.2.14. Sacral ventral rhizotomy:

Laminectomy was performed at the S1-S4 level and the spinal cord was exposed. The dura mater was cut longitudinally to expose the dorsal and ventral roots. The ventral roots of either side were sectioned after placing an ice cube on the ventral roots (Fig. 2.1D).

2.2.15. Nerve stimulation:

The central cut ends of left vagus (VAG) and LCN were stimulated with Grass SD9 stimulator using square wave pulses of stimulus strength of 60 Hz, 0.6 mS, 6V for 30 secs. The central cut end of the nerve was placed on a bipolar electrode soaked in liquid paraffine and then stimulated (Fig. 2.1C).

The peripheral cut ends of the sacral ventral roots at the S2-S4 level were split into 7-8 thick strands and these strands were stimulated separately with Grass SD9 stimulator.

2.2.16. Drug administration:

Different drugs were administered either intravenously or locally.
2.2.16.1. Intravenous administration:

Drugs were dissolved in normal saline so that the required dose was present in 0.5 ml. 0.5 ml drug solution was injected intravenously (through femoral catheter) and each injection was followed by 0.5 ml saline solution so that no drug remained in the cannula.

2.2.16.2. Local application:

Nicotine was diluted with normal (0.9%) saline solution to make 100 or 200 μg/ml solution. A cotton film of approximately 7-9 mm diameter was soaked in this solution and the film was placed on the epicardial surface of the left ventricle for 30-60 secs (Fig.2.1B). Then the cotton film was removed and the ventricular surface was washed with normal saline for at least 4 times. An interval of at least 20 minutes was given before another application. Lignocaine was also applied in a similar fashion.

2.3. Estimation of blood pressure or intravesicular pressure

Pen position, on disconnection of the pressure transducer with the arterial or bladder cannula indicates the "0" (zero) level of the pressure transducer. Calibration of the pressure transducer was performed by connecting the transducer to a "U" tube mercury manometer and pressure levels were recorded by inducing different pressures manually with the help of a saline filled syringe connected to the other arm of the "U" tube. A standard curve was drawn with these pressure heads against the recorder pen deflection. The B.P. or I.V.P. was calculated from this standard curve.

2.4. Statistical analysis

The initial normal contraction pressure was obtained by averaging peak pressures of 10 successive normal contractions of the urinary bladder. The intravesicular pressure (IVP) was recorded in mmHg. Change (maximum) of IVP during reflex contraction or relaxation was obtained from the difference between the initial peak pressure and the pressure during the reflex and expressed as mean % change ± SEM. Student "t" tests were performed with the IVP (absolute value) preceding and following LAD occlusion or nicotine application in control animals. And similar significance tests were also performed with the average percentage change in IVP or BP following LAD occlusion or nicotine application in control and experimental animals. Urine flow...
rate was expressed as average drops/min and significance of differences between control and experimental groups were tested using students "t" test for paired observations. P<0.05 was considered as significant and P<0.001 was considered as highly significant differences.

2.5. Drugs used

Anaesthetic ether (Kabra Drugs Ltd, India), α-chloralose (Koch-Light Lab., U.K), Acetylcholine chloride (Sigma Chemicals, U.S.A.), Adrenaline acid tartate (Burroughs Welcome, India), Atropine sulphate (Bengal Immunity, India), Lignocaine ("Xylocaine", Astra IDL, India), Nicotine ((-)Nicotine, Merck, Schuchardt), Phentolamine mesylate ("Regitine", Ciba-Geigy, U.K.), Propranolol hydrochloride (ICI, India), Bretylium tosylate (Sigma Chemicals, U.S.A.), Isoprenaline sulphate (Burroughs Welcome, India), Salbutamol Sulphate (Opec Innovations, India), Pancuronium bromide ("Pavulan", Infar India Ltd, India), Atenolol ("Aten 25", Kopran Limited, India), Hexamethonium bromide (Koch-Light Lab, UK), Guanethidine sulphate (Sigma Chemicals, U.S.A.), Captopril (Wockhardt Ltd., India).