1.4. Summary

Distension of the urinary bladder results in appreciable changes in the cardiovascular system in human subjects as well as in experimental animals (TAYLOR, 1974). There exists a great deal of uncertainty about the afferent mechanisms. Neurogenic factors were made responsible for the efferent mechanisms. Respiratory changes during the bladder distension received little attention. The present experiments were carried out to evaluate not only the cardiovascular changes but also the respiratory responses during the bladder distension. An attempt was made to evaluate the relative contributions of the pelvic and hypogastric nerves. A qualitative analysis of the contribution of humoral factors especially the renin-angiotensin system during the bladder distension was also carried out.

2. MATERIAL AND METHODS

2.1. Experimental animals

Preliminary experiments were performed on 34 dogs of either sex. Their weights ranged from 5.5 to 16.0 kg, with a mean value of 10.6 $\pm$ 2.2 kg. In another series of experiments 42 cats of either sex weighing between 1.9 to 4.1 kg ($\bar{X}$ = 2.8 ; $\pm$ 0.10) were used.

2.2. Anaesthesia

Dogs were anaesthetized by intravenous injection of freshly prepared α-glucochloralose (100mg·ml^{-1} of distilled water at 56°C). The dosage of the chloralose used was 80 - 100 mg·kg^{-1} of bodyweight. In some experiments, prior to chloralose, ether

anaesthesia was given to get sufficient cooperation from the dog while giving the intravenous injection. During the experiment, when it was necessary 10 - 15 mg chloralose·kg^{-1} of bodyweight was given intravenously.

In the experiments performed on cats, anaesthesia was primarly induced by 80 % O_2 and 20 % N_2O. Thereafter the animal was anaesthetized with intravenous injection of α-glucochloralose-urethane solution (0.5 g α-glucochloralose, 2,5 g urethane in 10 ml of distilled water at 56°C). For each kilogram of bodyweight 0.8 ml of this solution was injected, which gives a dosage of 40 mg chloralose and 200 mg urethane per kilogram of bodyweight. With this method one achieves a sufficiently light anaesthesia lasting for 7 - 8 hours. If the anaesthesia was found to be too superficial, then 6 - 12 mg of Nembutal (pentobarbitone sodium) was injected either intravenously or intraperitoneally.

2.3. Operation

2.3.1. Venous, arterial and tracheal cannulations

After the induction of anaesthesia the animal was placed on an operation table. The right femoral vein was cannulated with a polyethylene catheter. The tip of the catheter was placed in the inferior vena cava approximately at the level of the diaphragm. Infusion of saline and drugs could be made through this cannula. In some experiments from the same catheter venous pressure in the inferior vena cava was also measured. The left femoral vein was also cannulated in some experiments in which simultaneous measurement of the venous pressure distal to the urinary bladder was done. In the neck the trachea was exposed. At the level of the 2nd. and 4th. tracheal rings, the trachea was cannulated. In the experiments on dogs a carotid artery and in the cats a femoral artery were cannulated (tip lying in the aorta) for the purpose of measuring the arterial pressure.

2.3.2. Exposure and preparation of the bladder, the ureter and the urethra

The method of exposing the urinary bladder was essentially the same both in dogs and cats. The urinary bladder was exposed through a lower mid-line abdominal incision. Blood vessels and nerves pass over to the bladder wall along with the terminal part of the ureter. So as to avoid the damage of these blood vessels and the bladder nerves, the ureters were always tied about 3 to 4 cm above their termination. Two ligatures were used between which the ureters were cut. The proximal ends of the ureters were cannulated. The thin serous membrane of the peritoneum connecting the bladder to the anterior abdominal wall was then cut between the ligatures. The urethra was cleared of periurethral tissue and the distal part of the urethra was ligated. A rigid polyethylene cannula was introduced into the proximal end of the urethra. During the operation procedure, the bladder was covered with cotton gauze soaked in warm ringer solution or saline.

2.3.3. Preparation of the bladder nerves

These experiments were performed on cats only. After exposing the urinary bladder through a midline abdominal incision, the intestinal organs were gently placed on one side and covered with cotton gauze soaked in ringer or saline solution. The inferior mesenteric ganglion was located and the hypogastric nerve joining the ganglion was separated from the adjoining tissues, until to the close proximity of the bladder. Just near the bladder, the hypogastric nerve was cut between two ligatures. The same procedure was also carried out on the other side. The cut central end of one hypogastric nerve (either the right one or the left one) was used for electrical stimulation. Similarly, pelvic nerves were isolated on both sides close to the bladder neck. About 2 to 3 cm length of the nerves was separated from the surrounding bladder vessels and tissue. It was made sure that these nerves which were isolated were not joining the hypogastric nerves. After

confirming this point, the nerves were cut between ligatures and the cut central end of one nerve (either the right or the left one) was used for the purpose of electrical stimulation.

2.3.4. Isolation of the sino-aortic nerves

After cannulating the trachea the incision was extended further as rostrally as possible. The larynx and the oesophagus were separated from the adjoining blood vessels and nerves. Between ligatures, the larynx and oesophagus were cut and retracted in the rostral direction through an incision in the floor of the mouth.

The carotid sinus nerve was identified at the point where it joins the glossopharangeal nerve. A thread was placed around the nerve. Similarly, the aortic nerve was identified when it deviates from the vagus at the point of junction of the superior laryngeal nerve. It was dissected clear and a loop of thread was placed around the nerve. Cotton soaked with ringer solution was placed on the nerves to prevent them from drying. During the course of the experiment these nerves were cut bilaterally. The completion of the sinoarotic denervation was confirmed by the absence of the arterial pressor response to clamping the carotid artery and also the failure of hyperventilation after intravenous injection of 100 µg of sodium cyanide.

2.3.5. Isolation of the renal nerves and adrenelectomy

The abdominal incision was extended further in rostral direction. The abdominal organs were gently placed towards one side. The renal plexus around the renal artery was separated carefully from the renal artery. The renal nerves were isolated and a loose ligature was placed around them. Later, during the experiment renal nerves were bilaterally cut.

In the experiments where the efferent renal nerve activity was

recorded, the left kidney was exposed by a retroperitoneal approach. With the help of a dissecting microscope, a renal nerve of 2 - 3 cm was dissected free from the surrounding tissue. Then this nerve was cut very close to the kidney. By using fine forceps the nerve was desheathed. The desheathead nerve was tied with a fine silk thread.

With careful blunt dissection the adrenal artery and vein were isolated. Double ligatures were used to ligate these vessels. After the ligation of these vessels the adrenals were bilaterally removed.

2.3.6. Isolation of the phrenic nerve

The upper roots of the phrenic nerve were identified low in the neck on the left side, as they emerged at the lateral border of the anterior scalenus muscle. Then the brands of the nerve was dissected free from the tissue. The desheathed central end of the cut nerve was used for recording the electrical activity.

2.4. Artificial respiration

In some experiments the cats were paralysed by intravenous injection of Flaxedil (gallamin triethyliodide) with a dose of 6 $mg \cdot kg^{-1}$ of body weight. Then the cats were artificially ventilated with a Starling-pump.

The respiratory frequency (12 - 18 min^{-1}) and respiratory volume (30 - 50 ml) were so adjusted that the endexpiratory P_{ECO_2} was maintained at about 30 - 40 Torr.

2.5. Distension of the urinary bladder

2.5.1. Distension of the bladder with saline solution

The polyethylene cannula with which the urethra was catheterized was connected to a T-tube. One arm of the T-tube was now connected through a Y tube to a reservoir containing freshly prepared 0.9 % (W/V) sodium chloride solution. The side arm of the 'T' tube was connected to a mercury manometer in the experiments with dogs and to a strain gauge transducer (Statham) in the experiments in cats for measuring the intravesical pressure. The other limb of the Y tube was used for emptying the bladder. The saline solution coming from the reservoir to the bladder, was kept warm (approximately 37°C) by passing it through a perfused water jacket. The height of the saline reservoir was adjusted to give a pressure head of 80 Torr in the experiments on cats and 90 Torr in experiments involving dogs. Usually the filling rate for bladder distension was about 45 - 50 $ml \cdot min^{-1}$ (dogs) and 75 - 80 $ml \cdot min^{-1}$ (cats). With this method of distension one achieves an intravesical pressure of about 40 - 80 Torr. After distending the bladder for a desired period of time, the bladder volume was measured by emptying it into a measuring cylinder. In some experiments the urinary bladder was exteriorized from the abdominal cavity and distended keeping the vascular and nervous supply intact. The exteriorized bladder was prevented from drying by covering it with cotton gauge soaked in ringer solution. During this exteriorized distension, the incision of the abdominal cavity was closed.

2.5.2. Pneumatic distension of the urinary bladder

In some preliminary experiments on dogs, after exposing the bladder an incision was made in the bladder. A rubber tube with a balloon attached to its end was introduced into the bladder. This rubber tube was connected to a 'T' piece. One end of which was connected to a sphygmomanometer bulb and the other end to the sphygmomanometer itself to measure the intraluminal pressure

inside the urinary bladder. The balloon inside the bladder was distended until intraluminal pressure of 20 to 120 Torr resulted.

2.6. Electrical stimulation

2.6.1. Electrodes

For the purpose of electrical stimulation bipolar electrodes made of stainless steel were used. The distance between the two electrodes was 2 mm and the thickness of each electrode was 0.2 mm. The stimulating electrode towards the central nervous system has always been the cathode. The electrode with the nerve on it was covered with paraffin oil.

2.6.2. Stimulating apparatus

A Grass stimulator (model S 88) was used to deliver the electrical stimulus. The stimulator was connected to a Grass stimulus isolation unit. The stimulus parameters were continuously measured and monitored on a cathode ray oscilloscope.

2.6.3. Stimulus parameters

Stimulus parameters used for the electrical stimulation of the afferents from the bladder were 4 - 5 V, 10 - 20 imp.sec^{-1} and 2 ms. These parameters were chosen because they were found to elicit maximal cardiovascular and respiratory responses.

2.7. Recording and measurement of parameters

2.7.1. Recording and measurement of parameters in dogs

In the experiments on dogs all the registrations were done on a Palmer kymograph. The speed of the revolving drum was adjusted either at 1.6 $mm \cdot sec^{-1}$ or 0.64 $mm \cdot sec^{-1}$. The speed of 1.6 $mm.sec^{-1}$ was found appropriate to count the heart frequency from the arterial pressure.

1. For the purpose of measuring the arterial and intravesical pressures mercury manometers were used, while for the venous pressure measurement water manometers were used.
2. The heart frequency was measured either by counting from E.C.G. (lead II) or from the systolic pressure fluctuations.
3. Respiratory frequency was measured by connecting the tracheal cannula to a Marey tambour.
4. The rectal temperature of the animal was measured by means of a thermometer.

2.7.2. Recording and measurement of the parameters in cats.

In the experiments with cats all the measurements were recorded on a Beckman multichannel recorder (Type R Dynograph).

1. The recording of the arterial and the intravesical pressures were achieved by Statham strain gauge transducers.
2. The heart frequency triggered from the arterial pressure signal was fed to a frequency measurement counter (Luttmann and Mückenhoff, 1974).
3. For the purpose of measuring the tidal volume and the respiratory frequency, the tracheal cannula of the animal was connected to an O_2 supplied semi-closed system. CO_2 produced by the animal was absorbed by soda-lime. The gas in the system was circulated by the use of a pump. The respiratory tidal volume and the respiratory frequency were measured with a Krogh spirometer to which an inductive transducer was connected.

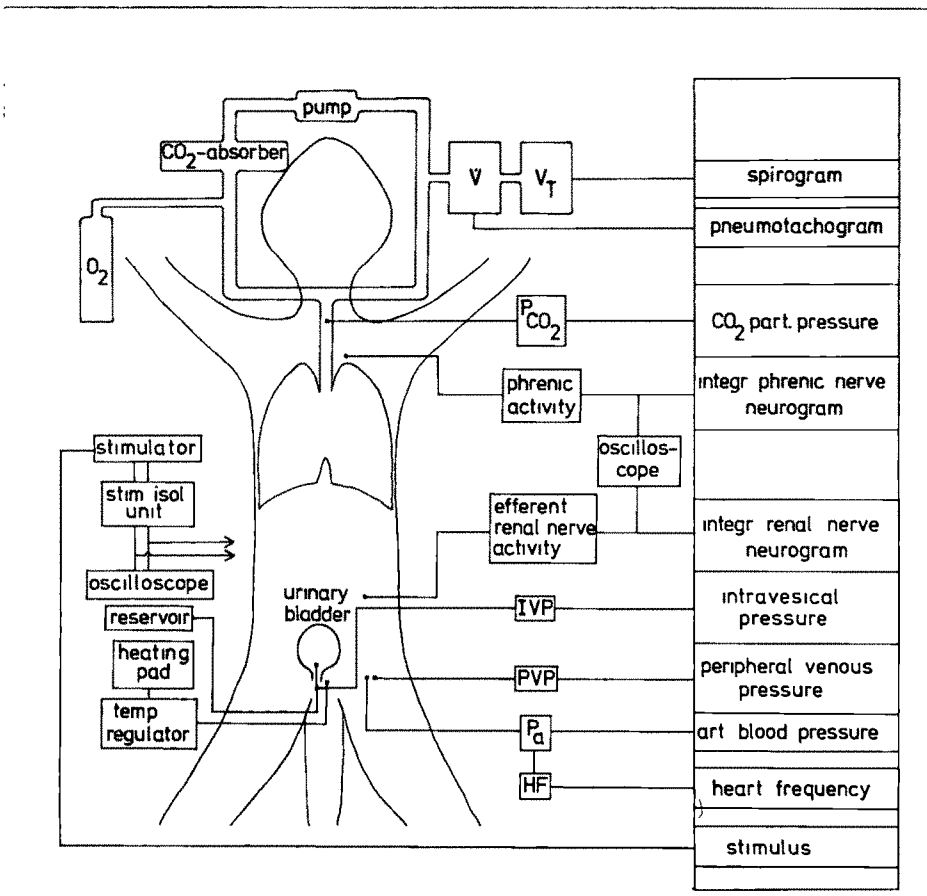

Figure 1: Schematic representation of the experimental set up

4. For the determination of the end expiratory CO_2 ($P_{E\ CO_2}$) an infrared CO_2 analyser (Type Hartmann and Braun Uras 4) was connected to the tracheal cannula. The sample was collected continuously by a steel needle with a high flow resistance. The flow was kept constant at 40 $ml \cdot min^{-1}$.

5. The rectal temperature of the animal was measured with a temperature meter (Mettler TM 16) and adapted by a heating pad, automatically holding the body temperature of the animal at $37 \pm 1.0^{\circ}C$.

6. The electrical activities of the phrenic and renal nerves were recorded by means of a preamplifier on a two channel storage oscilloscope (Textronix F 119) with a filter range between 100 Hz and 1, 5, or 10 kHz.

7. The neurograms were integrated with a R-C integrator. The time constants of the integrator used were 50 or 100 ms.

8. During the electrical stimulus the stimulus marking was recorded by means of an automatic marker.

2.8 Preparation and infusion of the drugs

Two types of angiotensin II antagonists were used. They are 1-sarcosine-8-alanine angiotensin II (Beckman) and 1-sarcosine-8-leucine angiotensin II. Stock solutions of the antagonists were prepared by dissolving 5 mg of the peptide in 1 ml of 0.1 M acetic acid.

The slow infusion of the antagonist was performed by using an infusion pump. Angiotensin II (Hypertensin, CIBA) and norepinephrine (Arterional, Hoechst AG) were used as agonists, to test the efficacy and specificity of the antagonists.

2.9. Statistical analysis

For the purpose of statistical calculation, the results were analysed in the following way:

1. Arithmetic mean: $\bar{X} = \frac{\Sigma X}{n}$ where ΣX equals the sum of individual results and n is the number of observations.

2. Standard deviation: $s = \pm \sqrt{\frac{\Sigma (X_i - \bar{X})^2}{n - 1}}$

3. Standard error of the mean: $Sm = \frac{s}{\sqrt{n}}$

4. Fischers t-test: $t_{(n_1+n_2-2)} = \frac{[\bar{X}_1 - \bar{X}_2]}{s_D}$

   where $\bar{X}_1$ and $\bar{X}_2$ are the arithmetical means of 2 populations and $s_D = \pm\sqrt{s_1^2 + s_2^2}$.

   The probability P of the Null hypothesis was taken from the diagram of PÄTAU (1943).