

CHAPTER - 3

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### 3. METHODS

#### 3.1. IN VIVO STUDIES :

##### 3.1.1. ACUTE EXPERIMENTS :

##### 3.1.1.1. EFFECTS OF ACUTE ADMINISTRATION OF CdCl<sub>2</sub> ON RAT BLOOD PRESSURE

Female albino rats (Haffkine strain) 200-250 g were used. Rats were anaesthetized with pentobarbitone sodium (40 mg/kg, i.p.) and tracheostomy was performed. The blood pressure was measured directly through the left common carotid artery by Statham Pressure Transducer (Sanborn model P23 AA); and recorded on calibrated Twin Viso Recorder (Sanborn). The femoral vein was cannulated with a needle (24 No. stylus removed) connected to a fine polyethylene catheter for the injection of drugs. Heparin (100 units/ml) solution was filled on the dome of the transducer and also in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting. The animals were divided into different groups containing 4-6 rats each. The rats of each group having undergone the above mentioned surgical procedures, were subjected to different experiments as follows:—

For the first series of experiments, CdCl<sub>2</sub> (0.1, 0.5 and 1 mg/kg) dissolved in 0.9% NaCl was administered

intravenously to a group of female rats. Continuous tracing of arterial blood pressure was made on the recorder. Each dose was administered at intervals of 20 minutes. The effect of saline 0.2 ml (i.v.) was recorded for 15 min before the administration of CdCl<sub>2</sub>.

For the second series of experiments, CdCl<sub>2</sub> (0.5 and 1 mg/kg) was administered intraperitoneally and blood pressure was recorded. 0.2 ml of 0.9% NaCl was also injected intraperitoneally 30 min before CdCl<sub>2</sub> administration. Each dose of CdCl<sub>2</sub> was administered at intervals of 30 minutes.

3.1.1.2. IN VIVO VASCULAR REACTIVITY TO AGONISTS AFTER CdCl<sub>2</sub> ADMINISTRATION:

In the next series of experiments, the vascular reactivity to various agonists was studied on the rat blood pressure in the presence of CdCl<sub>2</sub>.

To one group of animals, noradrenaline (NA) (0.5, 1 and 2 µg/kg) angiotensin II (ANG) (50, 100 and 200 ng/kg) and to another group isoprenaline (0.5 and 1 µg/kg) and acetylcholine (ACh) (50 and 100 ng/kg) were administered intravenously before and after the acute intravenous administration of CdCl<sub>2</sub> (1 mg/kg). Each dose of the agonist was administered at intervals of 10 minutes.

To the next group of animals, blood pressure effects of NA (0.5, 1 and 2  $\mu\text{g}/\text{kg}$ , i.v.) and ANG (50, 100 and 200  $\text{ng}/\text{kg}$ , i.v.) were recorded before and after the acute intraperitoneal administration of  $\text{CdCl}_2$  (1  $\text{mg}/\text{kg}$ ).

3.1.1.3. EXPERIMENTS TO INVESTIGATE MECHANISM OF ACUTE PRESSOR EFFECT TO  $\text{CdCl}_2$  :

To investigate the mechanism of acute blood pressure response to intravenous and intraperitoneal administration of  $\text{CdCl}_2$ , various inhibitory test substances were used. The protocols are as follows.:

A group of female rats were administered phentolamine (5  $\text{mg}/\text{kg}/\text{min}$ , i.v.) dissolved in 0.9% NaCl. The blood pressure response to  $\text{CdCl}_2$  (1  $\text{mg}/\text{kg}$ , i.v.) was elicited before and after phentolamine challenge. NA (1  $\mu\text{g}/\text{kg}$ , i.v.) was administered before and after phentolamine to evaluate the blockade of  $\alpha$  receptor.

To the next group of female rats,  $\text{CdCl}_2$  (1  $\text{mg}/\text{kg}$ ) was administered intravenously before and after hexamethonium (10  $\text{mg}/\text{kg}$ , i.v.) dissolved in 0.9% NaCl. DMPP (100  $\mu\text{g}/\text{kg}$ , i.v.) was administered before and after hexamethonium to evaluate the blockade of ganglia.

Acute reserpinization was carried out according to the method of Oliver and Shoji (1967). Reserpine (5  $\text{mg}/\text{kg}$ ) was

given intraperitoneally to a group of female rats. Twenty-four h after a single dose of reserpine, the acute blood pressure response to intravenous  $\text{CdCl}_2$  (1 mg/kg) was elicited. Tyramine (TYR) (100  $\mu\text{g}/\text{kg}$ ) was also administered intravenously to study the extent of reserpinization. Control responses to  $\text{CdCl}_2$  and TYR were studied in a separate group of female rats.

Similarly  $\text{CdCl}_2$  (1 mg/kg) was administered intravenously before and after propranolol (2 mg/kg, i.v.) to a group of female rats. Propranolol was dissolved in 0.9% NaCl. Isoprenaline (1  $\mu\text{g}/\text{kg}$ , i.v.) was administered before and after propranolol to study the extent of blockade of beta-receptors.

To another group of female rats,  $\text{CdCl}_2$  (1 mg/kg) was administered intravenously before and after atropine (1 mg/kg, i.v.) dissolved in 0.9% NaCl. ACh (100 ng/kg, i.v.) was administered after atropine to evaluate the blockade of muscarinic receptors.

Indomethacin (20 mg/kg) was given intraperitoneally in the form of suspension to a group of female rats. The suspension was prepared first by adding traces of Tween-80 to the drug and later made up to the required volume by adding 0.9% NaCl. One hour after the administration of indomethacin,  $\text{CdCl}_2$  (1 mg/kg) was given intravenously and

the effects on blood pressure were recorded. Control response to  $\text{CdCl}_2$  (1 mg/kg, i.v.) was recorded 20 min before indomethacin administration in the same animals. The effect of vehicle on  $\text{CdCl}_2$  response was studied in a separate group of animals.

The effect of calcium channel blockers was studied on the  $\text{CdCl}_2$ -induced pressure responses. Verapamil (0.5, 1 and 2 mg/kg, i.v.) dissolved in 0.9% NaCl, was given slowly at a rate of 0.2 ml/min to a group of female rats.  $\text{CdCl}_2$  (1 mg/kg) was given intravenously before and after the verapamil challenge. Similarly another calcium channel blocker, nifedipine (0.25 and 0.5 mg/kg) was given intravenously to a group of female rats. Nifedipine was dissolved in polyethylene glycol-400 (PEG-400) ethanol and saline in the ratio of 15:15:70 and was administered intravenously at a rate of 0.2 ml/minute.  $\text{CdCl}_2$  (1 mg/kg) was administered intravenously before and after nifedipine. A separate set of animals was used to study the effect of vehicle on the responses to  $\text{CdCl}_2$ .

In the next series of experiments, group of female rats were used to investigate the mechanism of acute blood pressure response to intraperitoneal administration of  $\text{CdCl}_2$ . Therefore, the effect of acute intraperitoneal administration of  $\text{CdCl}_2$  (1 mg/kg) was studied after phentolamine (5 mg/kg, i.v.), hexamethonium (10 mg/kg, i.v.), reserpine (5 mg/kg,

i.p.), propranolol (2 mg/kg, i.v.), indomethacin (20 mg/kg, i.p.), verapamil (0.5 and 1 mg/kg, i.v.) and nifedipine (0.25 and 0.5 mg/kg, i.v.).

Phentolamine, hexamethonium, propranolol and verapamil and nifedipine were injected intravenously 10 to 30 min before intraperitoneal administration of  $\text{CdCl}_2$  (1 mg/kg). Reserpine was administered to a group of rats 24 h before the effect of  $\text{CdCl}_2$  was elicited. Indomethacin was given intraperitoneally 1 h before the intraperitoneal administration of  $\text{CdCl}_2$ . The control response to intraperitoneal administration of  $\text{CdCl}_2$  was studied in a separate group of rats. Necessary precautions were taken to prevent the decomposition of nifedipine due to light.

### 3.1.2. CHRONIC EXPERIMENTS :

#### 3.1.2.1. EFFECT OF CHRONIC ADMINISTRATION OF $\text{CdCl}_2$ ON RAT BLOOD PRESSURE :

Female albino rats (Haffkine strain) weighing 200-250 g were used.  $\text{CdCl}_2$  was added to deionized water in the concentration of 0, 5, 25 and 100 ppm ( $\mu\text{g}/\text{ml}$ ) and was substituted for drinking water. After 4 or 8 weeks of consumption the systolic blood pressure was measured from the rat tail using B.P. recorder\* (Model 8005, UGO BASLE, Italy, measurement

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\* This indirect method of measurement of blood pressure was carried out in Sarabhai Research Centre, Baroda. However, this instrument could not be used for further studies. Hence in other protocols, the blood pressure was measured in anaesthetized rats directly through the carotid artery.

*Basile*

and recording of blood pressure in unanaesthetized animals).

To another group of female rats, CdCl<sub>2</sub> (0.1, 0.5 and 1 mg/kg, i.p.) was administered daily for two weeks and weight matched control group of animals received daily an intraperitoneal injection of saline (0.2 ml) for a similar period. After completion of treatment, the animals of all groups were anaesthetized with sodium pentobarbitone (40 mg/kg, i.p.) and the blood pressure was measured as described under protocol 3.1.1.1. (Acute experiments).

#### 3.1.2.2. VASCULAR REACTIVITY TO AGONIST IN TREATED ANIMALS :

Control animals and groups treated with chronic CdCl<sub>2</sub> were used to the following experimental protocols.

Effects of intravenous administration of NA (0.5, 1 and 2 µg/kg), ANG II (50, 100 and 200 ng/kg), ACh (50 and 100 ng/kg) and isoprenaline (0.5 and 1 µg/kg) were observed in control animals and in those chronically treated with CdCl<sub>2</sub> (0.1, 0.5 and 1 mg/kg/day, i.p., for two weeks).

#### 3.1.2.3. EXPERIMENTS TO EVALUATE THE MECHANISM OF CdCl<sub>2</sub> INDUCED HYPERTENSION :

##### 3.1.2.3.1. Bilateral adrenalectomy :

Bilateral adrenalectomy was performed as described by De Champlain and Van Ameringen (1972) in female rats anaes-

schedule, rats from each group were anaesthetized with pentobarbitone (40 mg/kg, i.p.) and the blood pressure was measured as mentioned previously. In adrenalectomized animals, the blood pressure effects of acute intraperitoneal or intravenous administration of CdCl<sub>2</sub> (1 mg/kg) were also observed.

#### 3.1.2.3.2. Chemical sympathectomy :

Chemical sympathectomy in adult female rats was achieved by using guanethidine (Johnson and O'Brien, 1976). The cadmium treated group of animals received a daily injection of 50 mg/kg, i.p. of guanethidine for three weeks followed by guanethidine and CdCl<sub>2</sub> treatment for two weeks. The untreated group of animals received daily injection of 50 mg/kg, i.p. of guanethidine alone for five weeks. The untreated group which received guanethidine alone for five weeks also received 0.2 ml of 0.9% NaCl for two weeks. The blood pressure of control animals (0.2 ml, 0.9% NaCl i.p., 2 weeks), animals treated with chronic CdCl<sub>2</sub> alone (1 mg/kg/day, i.p., for 2 weeks), untreated group (guanethidine alone) and treated animals (guanethidine + CdCl<sub>2</sub>) were compared. In guanethidine treated animals, TYR (100 µg/kg, i.v.) and NA (1 µg/kg, i.v.) were administered to confirm whether sympathectomy had been achieved or not. The acute intraperitoneal or intravenous effects of CdCl<sub>2</sub> (1 mg/kg), on the blood pressure of rats chronically treated with guanethidine were studied.

### 3.1.2.3.3. Treatment with captopril :

Captopril, an orally active inhibitor of angiotensin converting enzyme has been shown to have potent antihypertensive effects in rats (Laffan et al., 1978). Therefore, cadmium treated group of animals received daily injection of  $\text{CdCl}_2$  (1 mg/kg, i.p.) for two weeks along with daily oral administration of captopril (3 mg/kg) for two weeks. The untreated group of animals received captopril (3 mg/kg, p.o.) daily for two weeks. The blood pressures of control group (0.2 ml of 0.9% of NaCl, i.p., 2 weeks), those treated with chronic  $\text{CdCl}_2$  alone (1 mg/kg/day, i.p., two weeks), untreated group (captopril alone) and treated group (captopril +  $\text{CdCl}_2$ ) were compared. The acute intravenous or intraperitoneal effects of  $\text{CdCl}_2$  (1 mg/kg) on the blood pressure of rats chronically treated with captopril (3 mg/kg/day, p.o., two weeks) were studied.

### 3.1.2.3.4. Treatment with calcium channel blockers :

Verapamil, a calcium channel blocker, has been shown to reduce the blood pressure in DOCA-salt treated and other hypertensive animal models (Aguas<sup>and Nickerson,</sup> 1983). Therefore, to one group of female rats,  $\text{CdCl}_2$  (1 mg/kg) was given intraperitoneally daily for two weeks along with verapamil (30 mg/kg/day, p.o. dissolved in distilled water or 15 mg/kg/two times daily p.o. for two weeks). The untreated group

received verapamil alone for a similar period. After the completion of treatment the blood pressures of control group (0.2 ml of 0.9% NaCl/day, i.p., for two weeks), CdCl<sub>2</sub> (1 mg/kg/day, i.p., for two weeks) treated group, untreated group (verapamil alone) and treated group (verapamil and CdCl<sub>2</sub>) were compared. The effects of acute intravenous or intraperitoneal administration of CdCl<sub>2</sub> (1 mg/kg) on the blood pressure of animals chronically treated with verapamil were observed. Similarly nifedipine (10 mg/kg) was orally administered daily along with CdCl<sub>2</sub> (1 mg/kg, i.p.) for two weeks to one group of female rats. PEG-400 was used as a vehicle to dissolve nifedipine. The blood pressures of vehicle control group (vehicle 0.2 ml p.o. + 0.2 ml saline i.p., 2 weeks), CdCl<sub>2</sub> treated group (1 mg/kg/day, i.p., two weeks), untreated group (nifedipine alone) and treated group (nifedipine + CdCl<sub>2</sub>) were compared. The effects of acute intravenous or intraperitoneal administration of CdCl<sub>2</sub> (1 mg/kg) on the blood pressure of animals chronically treated with nifedipine were also studied.

### 3.2. IN VITRO STUDIES :

#### 3.2.1. ACUTE EXPERIMENTS :

The acute effect of CdCl<sub>2</sub> was studied on vascular smooth muscles of rats i.e. hindquarter preparation, isolated aorta, isolated portal vein and in non-vascular smooth muscles i.e. vas deferens and anococcygeus.

### 3.2.1.1. RAT HINDQUARTER PERFUSION EXPERIMENTS :

Perfusion of rat hindquarter was carried out as described by Brody et al. (1963) with partial modification. Female rats (200-250 g) were anaesthetized with pentobarbitone sodium (40 mg/kg, i.p.) and were placed dorsally on operating table. Jugular vein was cannulated with polyethylene tube to inject heparin (100 units/kg) for the prevention of clot. The abdomen was cut open by midline incision and the visceral tissues were pulled aside using tissue clamps. The descending abdominal aorta was located, cleaned and was separated from inferior vena cava. The aorta and inferior vena cava were cannulated with fine polyethylene catheters to allow the flow of the effluent. After cannulation of abdominal aorta and inferior vena cava, the upper part of the body, above the cannulation, was severed from the rest of the body. The abdominal aorta was flushed with warm carbogenated Krebs Henseleit (1932) until the effluent was free from blood. The polyethylene catheter in the abdominal aorta was connected by a pressure rubber tubing to one of the arms of a three-way (T) cannula. The tail of the cannula was connected by a rubber tube to the bottom of the Mariotte bottle which was kept at a height of 2 metres from the operation table. The carbogenated PSS in the Mariotte bottle was continuously allowed to perfuse at a rate of 2 ml/min at an inflow pressure of 50 mm Hg developed using

gravitational force. The resistance to perfusion flow through the hindquarter was monitored through one arm of the three-way tube which was connected by polyethylene tube to the pressure transducer (Model T. 301 pt 1107) recording on calibrated polyrite recorder (Medicare). The temperature of the perfusion fluid was maintained at 37°C by allowing the fluid to pass through spiral glass tube which was kept in an organ bath having thermostat and heating element. After 60-90 min of equilibrium period, the substances to be tested were injected in the volume of 0.02 - 0.05 ml using 0.05 ml Hamilton syringe inserted into the rubber tubing connecting abdominal aorta and the T cannula. The following experiments were conducted on this preparation:

$\text{CdCl}_2$  (0.5, 1 and 2 mg) was injected intra-arterially (into the rubber tube) and the change in perfusion pressure was recorded.

In the next set of experiments,  $\text{CdCl}_2$  (1 or 3  $\mu\text{g}/\text{ml}$ ) was added to the perfusion fluid which continuously perfused the hindquarter of rats. After 60 min of equilibrium period, the basal perfusion pressure was recorded and it was compared with control.

In one set of experiments, phentolamine (10  $\mu\text{g}/\text{ml}$ ) was added to the perfusion fluid which continuously perfused the hindquarter.  $\text{CdCl}_2$  (1 mg) was injected intra-arterially

and the perfusion pressure was recorded in the presence of phentolamine.

In the next set of experiments, reserpinization was carried out as mentioned in the acute in vivo experiments. Twentyfour h after acute reserpinization, the rats were prepared for hindquarter experiments.  $\text{CdCl}_2$  (1 mg) was injected intra-arterially and the change in perfusion pressure was recorded.

The acute effect of  $\text{CdCl}_2$  (1 mg) on the perfusion pressures was recorded in presence of verapamil (50 or 100  $\mu\text{g}/\text{ml}$ ) in the perfusion medium.

#### 3.2.1.2. ISOLATED RAT AORTA :

Rats (250-300 g) were sacrificed by a blow on the head and bled to death by cutting the neck vessels. Helically cut aortic strip was prepared from thoracic aorta and mounted in an organ bath of 40 ml capacity as described by Furchgott and Bhadrakom (1953). The bathing medium contained Krebs Henseleit solution of the following composition (mM): NaCl, 118.0; KCl, 5.4;  $\text{NaH}_2\text{PO}_4$ , 1.1;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.58;  $\text{CaCl}_2$ , 2.5;  $\text{NaHCO}_3$ , 25 and dextrose, 11.1. The solution in the organ bath was maintained at  $37 \pm 0.5^\circ\text{C}$  and (pH 7.4) gassed with carbogen. During 2 hours of stabilization period, the tissue was washed every 15 minutes.

Contractile responses to cumulative addition of agonists were recorded in the absence and presence of  $\text{CdCl}_2$  on smoked kymograph paper using isotonic frontal writing lever (X 15 and 2 g tension). The following agonists were studied on this tissue.

Cumulative dose-response curve of KCl ( $1.0 \times 10^{-2}\text{M}$  -  $3.16 \times 10^{-1}\text{M}$ ) in the absence and presence of  $\text{CdCl}_2$  ( $4.8 \times 10^{-8}\text{M}$ ,  $4.8 \times 10^{-7}\text{M}$ ,  $4.8 \times 10^{-6}\text{M}$  and  $1.44 \times 10^{-5}\text{M}$ ) was recorded. The tissue was exposed to  $\text{CdCl}_2$  for about 10-15 min before eliciting the dose-response curve of KCl. The contractile response to each dose of KCl was recorded for 90 seconds.

Cumulative dose-response curve of NA ( $6.9 \times 10^{-8}\text{M}$  -  $4.36 \times 10^{-6}\text{M}$ ) was recorded in the absence and presence of  $\text{CdCl}_2$  ( $4.8 \times 10^{-8}\text{M}$ ,  $4.8 \times 10^{-7}\text{M}$ ,  $4.8 \times 10^{-6}\text{M}$  and  $1.44 \times 10^{-5}\text{M}$ ). Contractile response to each dose of NA was recorded for 2 minutes.

The contractile effect of  $\text{CdCl}_2$  ( $4.8 \times 10^{-8}\text{M}$ ,  $4.8 \times 10^{-7}\text{M}$ ,  $1.44 \times 10^{-6}\text{M}$  and  $4.8 \times 10^{-6}\text{M}$ ) was also recorded.  $\text{CdCl}_2$  was added in the bath and contraction of the aorta was recorded for 2 min duration. Further the contractile response was tested in absence of calcium ion. The contractile response to  $\text{CdCl}_2$  was also tested in presence of phentolamine ( $1.0 \times 10^{-6}\text{M}$ ). The tissue was exposed to phentolamine in the bathing medium for 15 minutes.

### 3.2.1.3. PORTAL MESENTERIC VEIN :

The rat portal vein was cleansed and the longitudinal muscle was mounted in a 30 ml organ bath containing Krebs Henseleit solution maintained at  $37 \pm 0.5^\circ\text{C}$  and gassed with carbogen. Contraction to the agonists were recorded on isotonic frontal writing lever (X 15 and 1 g tension). The tissue was allowed to stabilize for one h during which the bathing medium was changed every 15 minutes.

Non-cumulative dose-response curve of KCl ( $1.0 \times 10^{-2}\text{M}$  -  $1.38 \times 10^{-1}\text{M}$ ) was recorded in absence and presence of  $\text{CdCl}_2$  ( $4.8 \times 10^{-7}\text{M}$ ,  $4.8 \times 10^{-6}\text{M}$ ,  $1.44 \times 10^{-5}\text{M}$  and  $4.8 \times 10^{-5}\text{M}$ ). Contractile response of each dose of KCl was recorded for 45 seconds.

Cumulative dose-response curve of NA ( $1.38 \times 10^{-7}\text{M}$  -  $8.7 \times 10^{-6}\text{M}$ ) was recorded in the absence and presence of  $\text{CdCl}_2$  ( $4.8 \times 10^{-7}\text{M}$ ,  $4.8 \times 10^{-6}\text{M}$  and  $4.8 \times 10^{-5}\text{M}$ ). The contractile response of each dose of NA was recorded for 1 minute. The contact period of  $\text{CdCl}_2$  to the tissue was 10-15 min before eliciting the dose-response curve of KCl and NA.

### 3.2.1.4. VAS DEFERENS :

Both vas deferentia of rat were promptly removed, desheathed, cleaned and mounted in a 30 ml organ bath containing Krebs Hukovic solution of the following

composition (mM): NaCl, 112.9; KCl, 4.69; CaCl<sub>2</sub>, 2.5; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.19; KH<sub>2</sub>PO<sub>4</sub>, 1.18; NaHCO<sub>3</sub>, 25 and dextrose, 11.1. The solution was maintained at 37±0.5°C and gassed continuously with carbogen. The contractile response of vas deferens was recorded on smoked kymograph with isotonic frontal lever (X 10 and 0.5 g tension). The tissue was allowed to equilibrate for 30 min during which the bathing medium was changed every 10 minutes. The following agonists were studied -

Dose-response curve of KCl (1.78 x 10<sup>-2</sup>M - 7.41 x 10<sup>-2</sup>M) was recorded in the absence and presence of CdCl<sub>2</sub> (4.8 x 10<sup>-9</sup>M, 1.44 x 10<sup>-8</sup>M, 4.8 x 10<sup>-7</sup>M and 4.8 x 10<sup>-6</sup>M). The contractile response of each dose of KCl was recorded for 60 seconds.

The dose-response curve of NA (2.75 x 10<sup>-7</sup>M - 3.47 x 10<sup>-5</sup>M) was recorded in the absence and presence of CdCl<sub>2</sub> (4.8 x 10<sup>-9</sup>M, 1.44 x 10<sup>-8</sup>M and 4.8 x 10<sup>-6</sup>M). Contractile response of each dose of NA was recorded for 60 seconds.

### 3.2.1.5. ANOCOCCYGEUS MUSCLE :

Healthy albino male rats of Haffkine strain weighing between 250-300 g were used in all experiments. The animals were stunned by a blow on the head and sacrificed by cutting

the blood vessels of neck. The abdominal mass was immediately opened. Both the anococcygeus muscles were located and then isolated in Petri dish containing carbogenated (5% CO<sub>2</sub> in 95% O<sub>2</sub>) Krebs physiological salt solution (PSS) of the following composition (mM): NaCl, 94.01; KCl, 4.69; CaCl<sub>2</sub>, 2.52; MgSO<sub>4</sub>, 0.46; KH<sub>2</sub>PO<sub>4</sub>, 1.7; NaHCO<sub>3</sub>, 25.0 and glucose, 10.75. The paired preparations were suspended in an organ bath (Gillespie, 1971) containing 30 ml of PSS at 37±0.5°C. The preparation was allowed to equilibrate for 30 minutes. The contractile responses to exogenously added agonists were recorded on smoked paper with isotonic frontal writing lever which was under 1.0 g tension and gave 8-fold magnification.

The dose-response curves of KCl ( $2.0 \times 10^{-2}M$  -  $9.0 \times 10^{-2}M$ ) and NA ( $1.0 \times 10^{-7}M$  -  $2.0 \times 10^{-5}M$ ) were recorded in the absence and presence of CdCl<sub>2</sub> ( $4.8 \times 10^{-6}M$ ;  $1.44 \times 10^{-5}M$  and  $4.8 \times 10^{-5}M$ ).

In another set of experiments, calcium was reduced to 25% and the dose-response curves of KCl and NA were elicited in the absence and presence of CdCl<sub>2</sub>.

### 3.2.2. CHRONIC EXPERIMENTS :

#### 3.2.2.1. HINDQUARTER PERFUSION :

Female rats were treated with CdCl<sub>2</sub> (0.1, 0.5 and 1 mg/kg/day, i.p.) for two weeks and they were used for

the following rat hindquarter perfusion experiments.

The basal perfusion pressure of the hindquarter preparations of control and treated rats were measured.

The change in perfusion pressure after the intra-arterial administration of NA (10,20 and 40 ug) in the hindquarter preparation of treated and control rats were measured.

3.2.2.2. IN VITRO SENSITIVITY OF VASCULAR AND NON-VASCULAR TISSUES OBTAINED FROM RATS CHRONICALLY TREATED WITH CdCl<sub>2</sub> :

The dose-response curves of NA and KCl on isolated aorta, portal vein, vas deferens and anococcygeus muscle were studied from the rats treated chronically with CdCl<sub>2</sub> (1 mg/kg/day, i.p., for two weeks).

3.3. BODY WEIGHT :

Female rats (200-250 g) were treated with CdCl<sub>2</sub> (0.1, 0.5 and 1 mg/kg/day, i.p.) for two weeks. The body weight before the beginning of the treatment, and two weeks after treatment, was observed and compared with control.

3.4. HISTOPATHOLOGICAL STUDIES :

Female rats weighing between 200-250 g were given CdCl<sub>2</sub> daily

intraperitoneally (1 mg/kg) for two weeks. Control rats received only 0.2 ml of 0.9% NaCl i.p. for a similar period. At the end of the treatment period, the rats were sacrificed and liver, kidney and heart excised, blotted free of blood and tissue fluids and fixed Bovins fluid. After a week, the tissues were washed thoroughly in repeated changes of 70% alcohol and then dehydrated in ascending grades of alcohol (70 to 100%). After dehydration the tissues were cleared in xylene and embedded in paraffin wax. Sections of 5 micron thickness were cut on a microtome and taken on glass slides coated with albumin. The sections were deparaffinated in xylene and downgraded through 100, 90, 70, 50, 30% alcohol and then finally in water. They were then stained with hematoxylin for 3-5 min and the staining was intensified by placing in running water. The hematoxylin stained sections were stained with eosin for 2 min and were then quickly passed through ascending grades of alcohol, cleaned in xylene and mounted on Canada balsam. The stained sections were observed under Carl-Zeiss photomicroscope and photographed.

### 3.5. CALCULATION OF pD<sub>2</sub> VALUES :

pD<sub>2</sub> values of the agonists were calculated as described by Ostil. (1963). Increases in pD<sub>2</sub> values was considered as a leftward shift of the dose-response curve and decrease in pD<sub>2</sub> values was considered as a rightward shift of dose-response curve.

### 3.6. STATISTICAL ANALYSIS :

Students 't' test was applied to determine the level of significance.  $P < 0.05$  was considered as statistically significant.

### 3.7. DRUGS AND CHEMICALS USED :

Acetylcholine chloride (BDH, London, England); angiotensin-II (Ciba-Geigy Pharmaceuticals Co., New Jersey, U.S.A.); atropine sulphate (BDH, London, England); cadmium chloride (Sarabhai Chemicals, Baroda, India); captopril hydrochloride (Squibb, New Jersey, U.S.A.); guanethidine sulphate (Ciba-Geigy of India Ltd., Bombay, India); hexamethonium chloride (Sarabhai Chemicals, Baroda, India); isoprenaline sulphate (Ward Blenkinsop and Co. Ltd., London, England); indomethacin (Cipla, Bombay, India); nifedipine (Sarabhai Chemicals, Baroda, India); (-); noradrenaline bitartrate (Sigma Chemicals, St. Louis, U.S.A.); pentobarbitone sodium (Apetho and Research Chemicals, Bombay, India); phentolamine

methane sulphate (Ciba-Geigy Pharmaceuticals Co., Basle, Switzerland); potassium chloride (Sarabhai M. Chemicals, Baroda, India); propranolol hydrochloride (Sarabhai Chemicals, Baroda, India); reserpine (Ciba-Geigy of India Ltd., Bombay, India); tyramine hydrochloride (Hoffman La-Roche and Co., Basle, Switzerland); verapamil hydrochloride (Boehringer-Knoll Ltd., Bombay, India).

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