CHAPTER - 4

4. RESULTS

4.1. IN VIVO STUDIES

4.1.1. ACUTE EXPERIMENTS

4.1.1.1. ACUTE EFFECTS OF $CdCl_2$ ON RAT BLOOD PRESSURE :

The acute intravenous administration of $CdCl_2$ (0.5 and 1 mg/kg) produced marked fall in blood pressure lasting for 60-90 seconds which was followed by an increase in blood pressure persisting for 10-15 minutes. At a lower dose (0.1 mg/kg) $CdCl_2$ produced a slight fall following a feeble rise in blood pressure (Fig. 1A and 2A).

The acute intraperitoneal administration of $CdCl_2$ (0.5 and 1 mg/kg) produced a pressor effect, without having any depressor component. This pressor effect occurred within 1 min of administration and persisted for about 25-30 minutes (Fig. 1B and 2B).

4.1.1.2. IN VIVO VASCULAR REACTIVITY TO AGONISTS :

The blood pressure responses to a low dose of NA (0.5 µg/kg) were significantly ($P < 0.05$) reduced after acute $CdCl_2$ (1 mg/kg, i.v.) administration, while those to higher doses were not modified (Fig. 3A). The blood pressure responses to different doses of ANG II, isopenaline and ACh were not modified (Fig. 3B and 4).

The blood pressure responses to different doses of NA and ANG were not modified after acute intraperitoneal administration of $CdCl_2$ (1 mg/kg) (Fig. 5A and 5B).

Fig. 1 : Tracing of arterial blood pressure of an anaesthetized (pentobarbitone sodium 40 mg/kg, i.p.) rat after intravenous (A) and intraperitoneal (B) injections of $CdCl_2$ (1 mg/kg). Abscissa indicates time in min following the injections and ordinate the blood pressure (mm Hg).

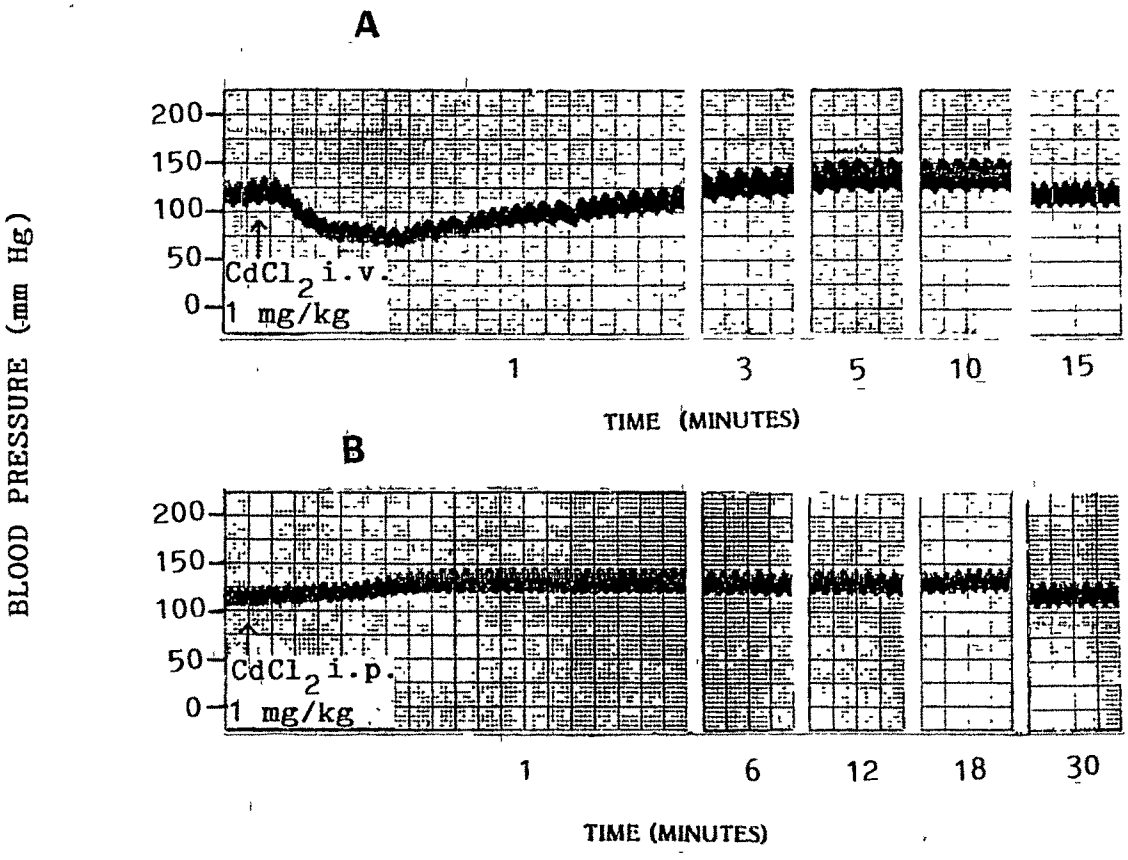
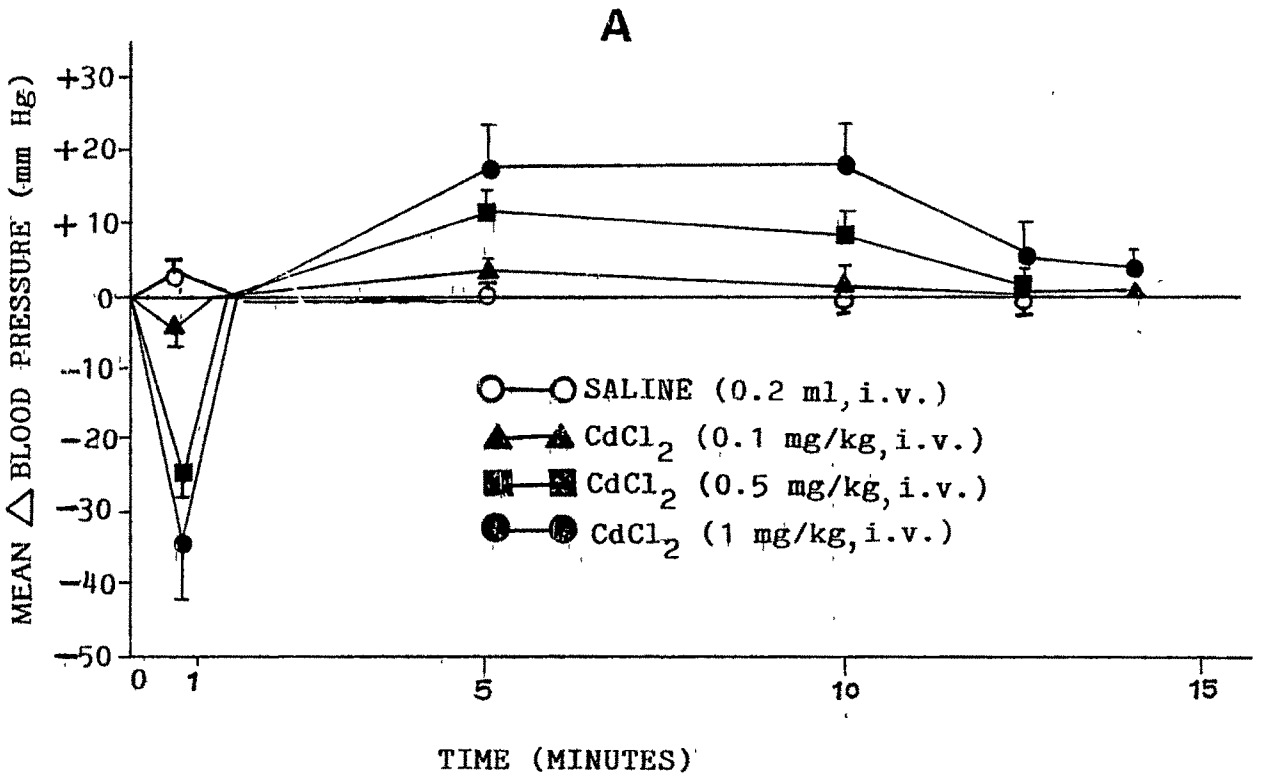

A
200
150
100
50
0
$CdCl_2$ i.v.
1 mg/kg
1
3
5
10
15
TIME (MINUTES)
B
200
150
100
50
0
$CdCl_2$ i.p.
1 mg/kg
1
6
12
18
30
TIME (MINUTES)
BLOOD PRESSURE (mm Hg)

Fig. 2 : Mean change in blood pressure of anaesthetized female rats with acute intravenous (A) and intraperitoneal (B) $CdCl_2$ injections. Abscissa indicates time in min following $CdCl_2$ injections and ordinate the change in blood pressure (mm Hg). Vertical lines represent SEM (n=5).


A
MEAN △ BLOOD PRESSURE (mm Hg)
+30
+20
+10
0
−10
−20
−30
−40
−50
0
1
5
10
15
SALINE (0.2 ml, i.v.)
CdCl2 (0.1 mg/kg, i.v.)
CdCl2 (0.5 mg/kg, i.v.)
CdCl2 (1 mg/kg, i.v.)
TIME (MINUTES)

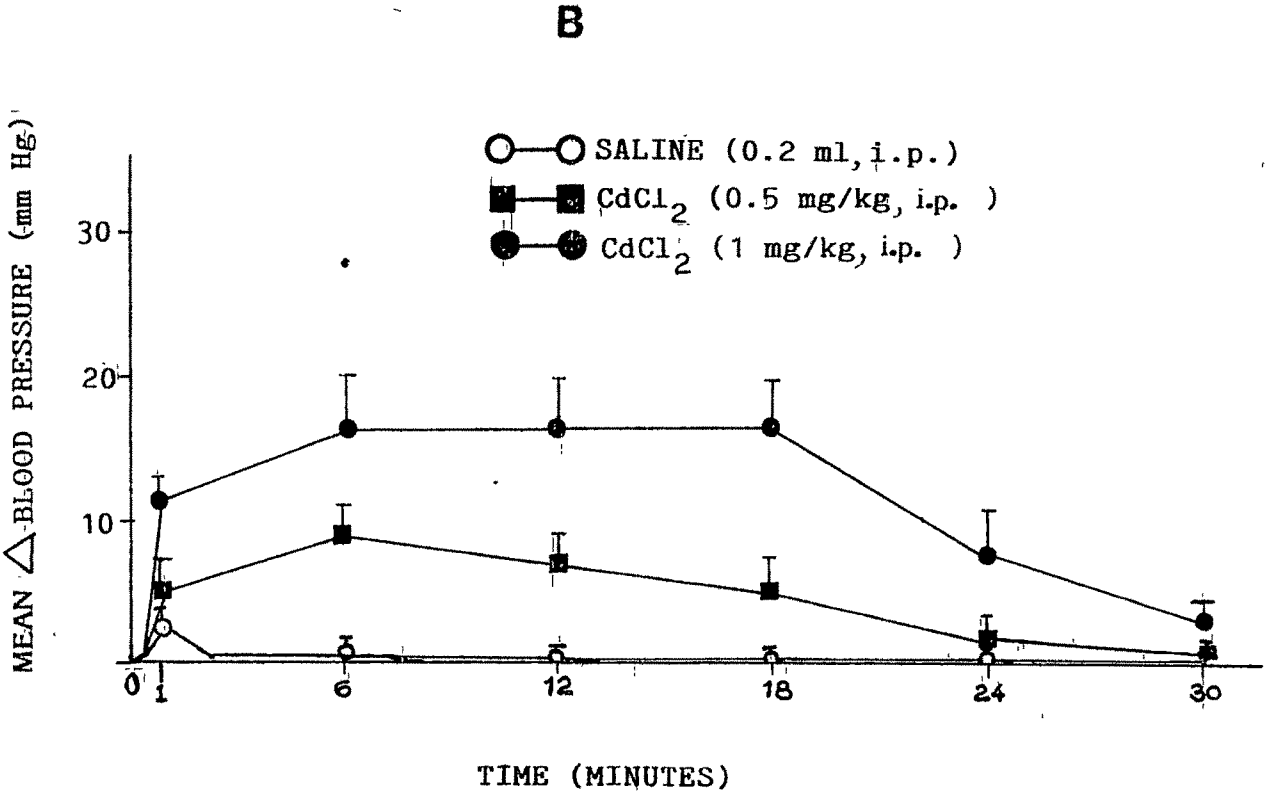

B
MEAN △ BLOOD PRESSURE (mm Hg)
30
20
10
0
1
6
12
18
24
30
SALINE (0.2 ml, i.p.)
CdCl2 (0.5 mg/kg, i.p.)
CdCl2 (1 mg/kg, i.p.)
TIME (MINUTES)

Fig. 3 : Mean change in blood pressure produced by acute intravenous injections of noradrenaline (NA 0.5, 1 and 2 µg/kg, A) and angiotensin II (ANG II 50, 100 and 200 ng/kg, B) in anaesthetized rats before and after intravenous $CdCl_2$ (1 mg/kg). Abscissa indicates doses of NA and ANG II and ordinate the mean change in blood pressure (mm Hg). Vertical lines represent SEM (n=5 to 6 for each observation). * $P < 0.05$ as compared with the corresponding control response).

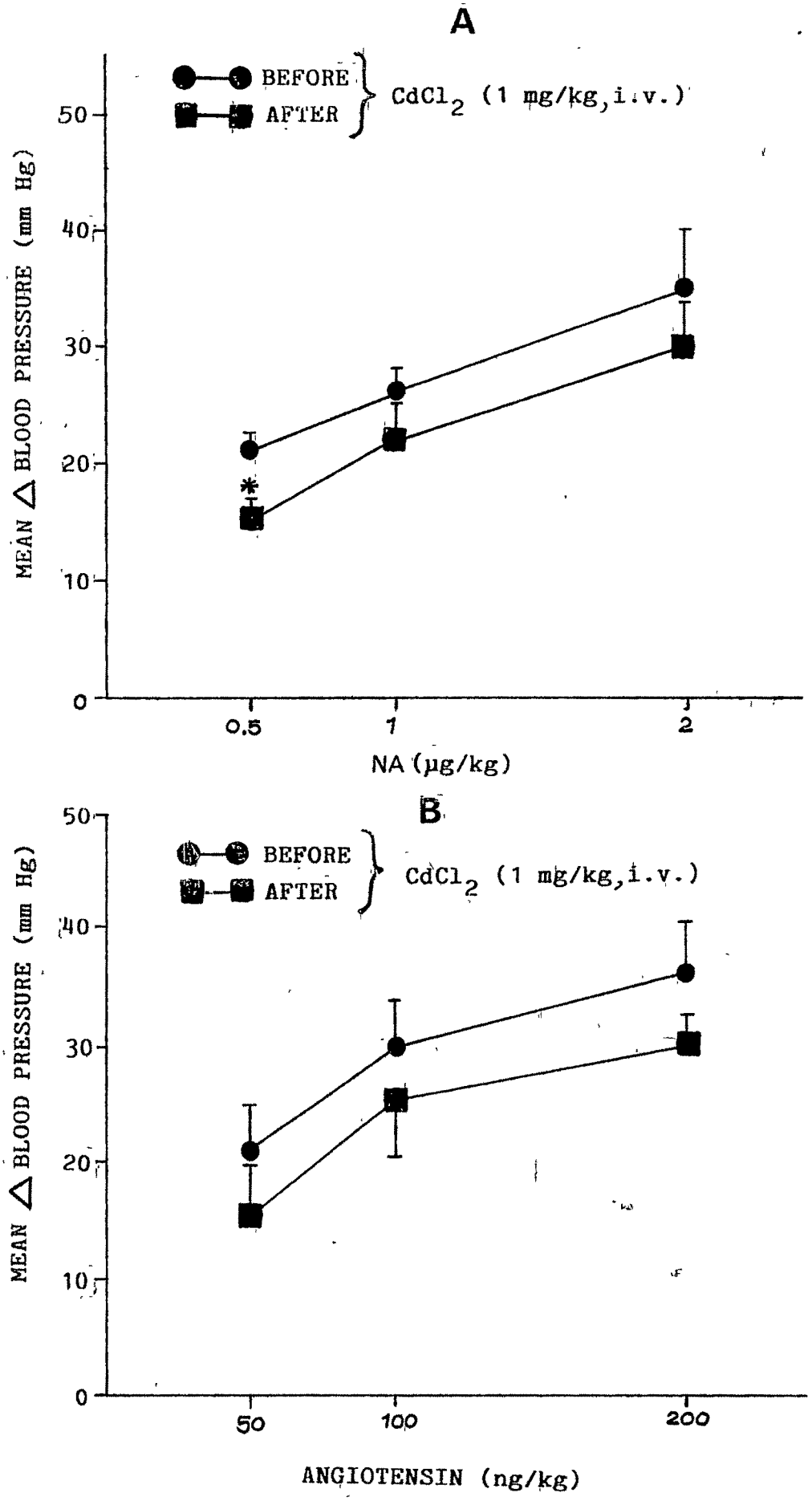

A
BEFORE
AFTER
CdCl2 (1 mg/kg,i.v.)
MEAN Δ BLOOD PRESSURE (mm Hg)
50
40
30
20
10
0
*
0.5
1
2
NA (µg/kg)
B
BEFORE
AFTER
CdCl2 (1 mg/kg,i.v.)
MEAN Δ BLOOD PRESSURE (mm Hg)
50
40
30
20
10
0
50
100
200
ANGIOTENSIN (ng/kg)

Fig. 4 : Mean change in blood pressure produced by acute intravenous injection of isoprenaline (0.5 and 1 μg/kg, A) and acetylcholine (ACh 50 and 100 ng/kg, B) in anaesthetized rats before and after intravenous $CdCl_2$ (1 mg/kg). Abscissa indicates doses of isoprenaline and ACh and ordinate the mean change in blood pressure (mm Hg). Vertical lines on histograms represent SEM (n=4 for each observation).

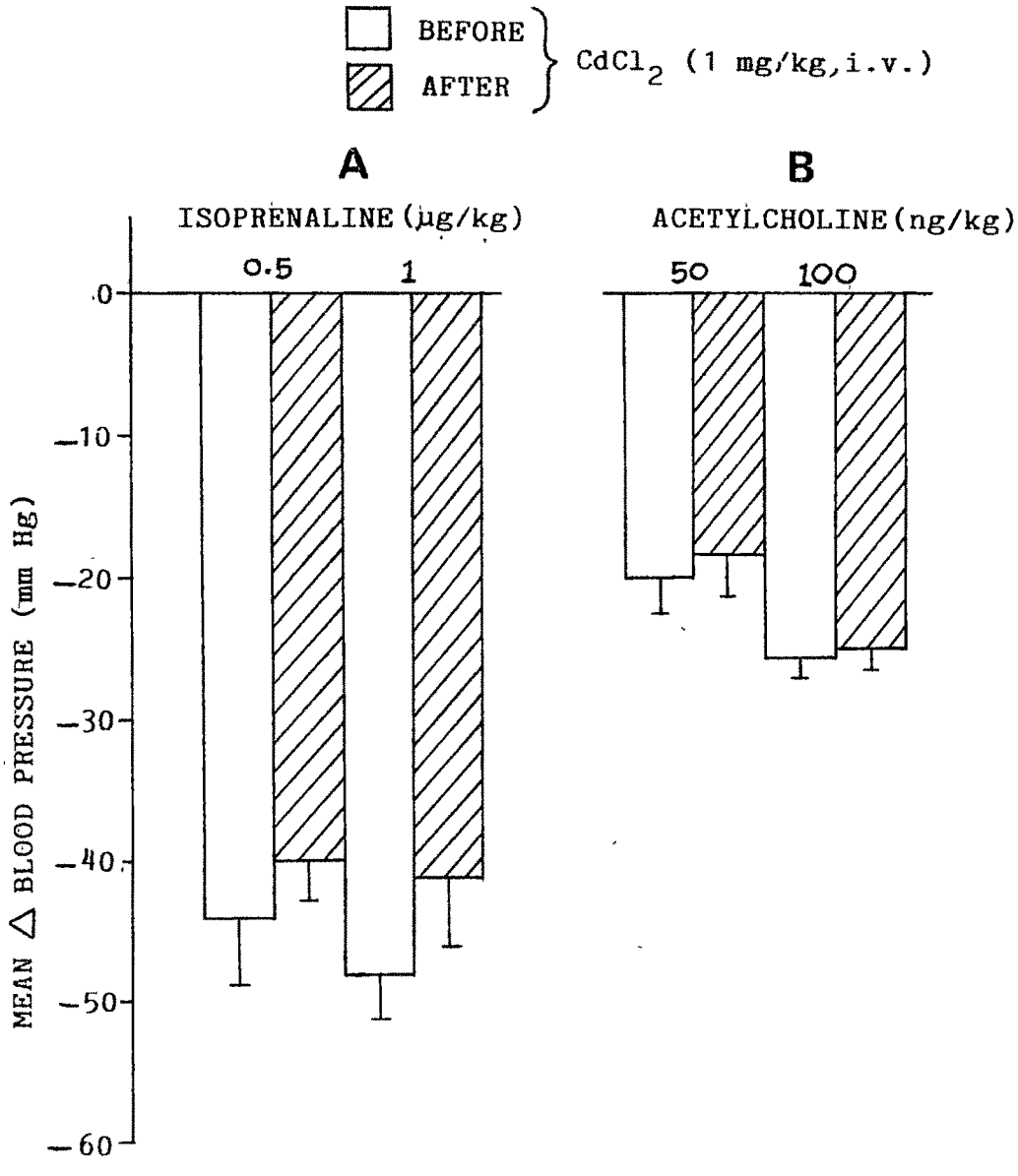
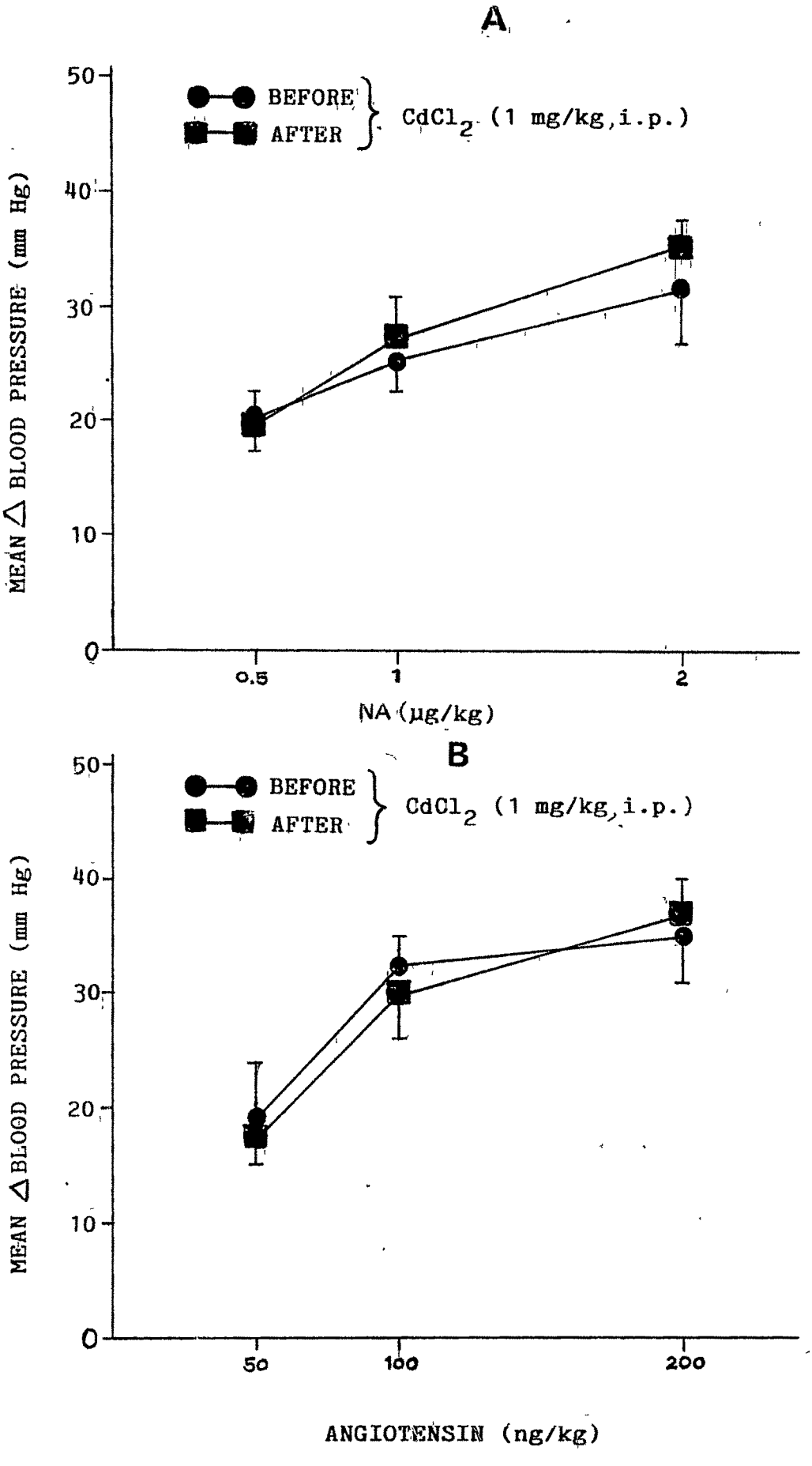

BEFORE
AFTER
CdCl2 (1 mg/kg, i.v.)
A
ISOPRENALINE (µg/kg)
0.5
1
B
ACETYLCHOLINE (ng/kg)
50
100
MEAN Δ BLOOD PRESSURE (mm Hg)
0
-10
-20
-30
-40
-50
-60

Fig. 5 : Mean change in blood pressure produced by acute intranevous injection of NA (0.5, 1 and and 2 μg/kg, A) and ANG II (50, 100 and 200 ng/kg, B) in anaesthetized rats before and after acute intraperitoneal $CdCl_2$(1 mg/kg). Abscissa indicates the doses of NA and ANG II and ordinate the mean change in blood pressure (mm Hg). Vertical lines represent SEM (n=5 for each observation).


A
BEFORE
AFTER
CdCl2 (1 mg/kg, i.p.)
MEAN Δ BLOOD PRESSURE (mm Hg)
50
40
30
20
10
0
0.5
1
2
NA (μg/kg)
B
BEFORE
AFTER
CdCl2 (1 mg/kg, i.p.)
MEAN Δ BLOOD PRESSURE (mm Hg)
50
40
30
20
10
0
50
100
200
ANGIOTENSIN (ng/kg)

4.1.1.3. EXPERIMENTS TO INVESTIGATE THE MECHANISM OF ACUTE PRESSOR EFFECT :

Phentolamine (5 mg/kg) given by a slow intravenous infusion produced a short-lived fall in blood pressure (Table VIII). The acute pressor response to an intravenous injection of $CdCl_2$ was not affected by phentolamine (Fig. 6A). However, the same dose of phentolamine completely blocked the NA response (1 µg/kg, i.v.) thereby confirming the blockade of alpha receptors.

Hexamethonium (10 mg/kg, i.v.) produced a depressor response (Table VIII). It did not modify the pressor response to intravenous administration of $CdCl_2$ (1 mg/kg) (Fig. 6B). However, complete blockade of the ganglia was confirmed since the same dose of hexamethonium completely antagonized the pressor response to DMPP (100 µg/kg, i.v.).

Acute reserpinization was achieved by administering reserpine (5 mg/kg) intraperitoneally. Twentyfour h later the pressor response to an intravenous injection of $CdCl_2$ was unaffected, while the pressor response to TYR (100 µg/kg, i.v.) was significantly blocked ($P < 0.001$) (Fig. 6C and 6D).

Propranolol (2 mg/kg, i.v.) produced a depressor response (Table VIII). It did not block the pressor response to $CdCl_2$ (Fig. 6E). However, the depressor response to

isoprenaline (1 μg/kg) was completely blocked by the same dose of propranolol suggesting blockade of beta receptors.

Atropine (1 mg/kg, i.v.) produced a depressor response (Table VIII). It did not affect either the depressor or the pressor effects of $CdCl_2$ (Fig. 6F). However, the muscarinic action of ACh (100 ug/kg) was completely blocked.

Indomethacin (20 mg/kg) given intraperitoneally, produced a fall of about 12-17 mm Hg (Table VIII). This effect persisted for about 10-15 minutes. $CdCl_2$ (1 mg/kg) was administered intravenously an hour after indomethacin injection. There was no significant change in the depressor or the pressor responses (Fig. 6G and 6H) to $CdCl_2$.

Verapamil (0.5, 1 and 2 mg/kg, i.v.) or nifedipine (0.25 and 0.5 mg/kg) produced a significant dose-related fall in blood pressure when given by slow intravenous infusions (Table VIII). The pressor response to intravenous administration of $CdCl_2$ (1 mg/kg) was significantly blocked ($P < 0.05$ and $P < 0.01$) by these drugs without modifying the depressor responses (Figs. 6I, J, K, L and M). However, when the vehicle (ethanol 15: PEG-400 15: 0.9% NaCl :70) was given intravenously, there was a slight rise in blood pressure of about 14 to 18 mm Hg (Table VIII). This effect persisted only for a few minutes. 0.2 ml of vehicle did not modify

the pressor or the depressor responses to $CdCl_2$ (1 mg/kg, i.v.) (Fig. 6N).

The pressor response to intraperitoneal injection of $CdCl_2$ (1 mg/kg) was not blocked after the administration of phentolamine (5 mg/kg, i.v.) (Fig. 7A), hexamethonium (10 mg/kg, i.v.) (Fig. 7B), reserpine (5 mg/kg, i.p.) (Fig. 7C), propranolol (2 mg/kg, i.v.) (Fig. 7D), or indomethacin (20 mg/kg, i.p.) (Fig. 7E). Verapamil (0.5 and 1 mg/kg, i.v.) or nifedipine (0.25 and 0.5 mg/kg, i.v.) significantly ($P < 0.05$ and $P < 0.01$) blocked the acute pressor response to $CdCl_2$ (1 mg/kg, i.p.) (Fig. 7F,G).

4.1.2. CHRONIC EXPERIMENTS :

4.1.2.1. EFFECTS OF CHRONIC ADMINISTRATION OF $CdCl_2$ ON RAT :

Chronic exposure of female rats to $CdCl_2$ dissolved in deionized water in concentrations of 5, 25 and 100 ppm for 4 weeks or 8 weeks did not elevate the systolic blood pressure (Table IX).

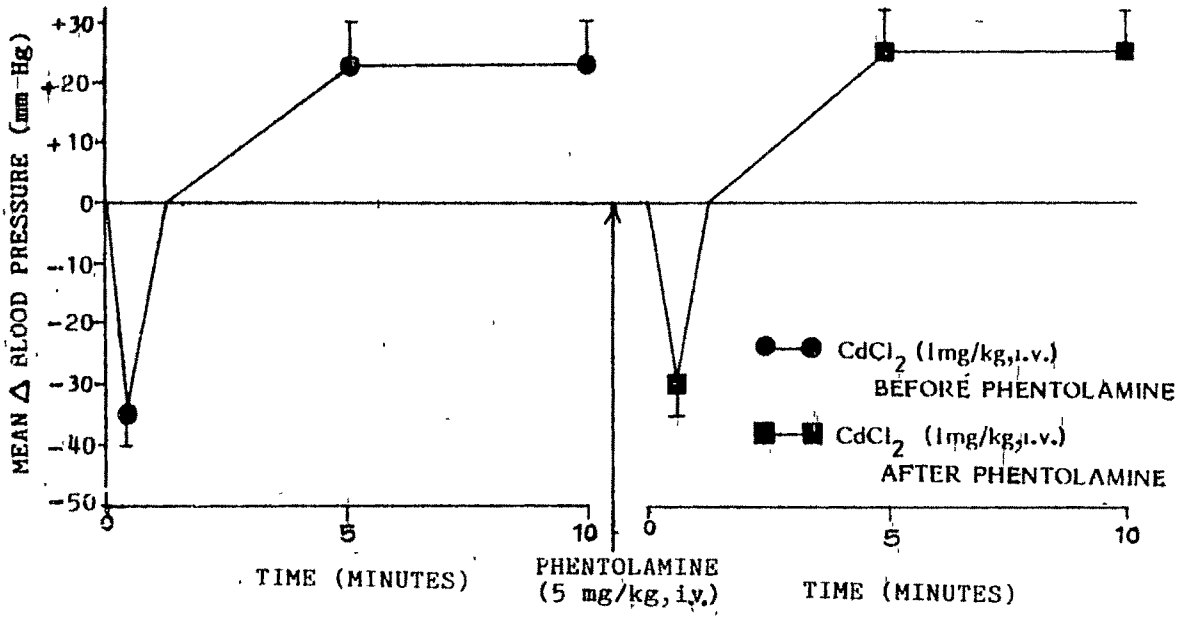
Female rats treated chronically intraperitoneally with $CdCl_2$ (0.5, 1 mg/kg/day) for two weeks exhibited significant ($P < 0.01$) elevation of arterial blood pressure. However, a lower dose (0.1 mg/kg/day) did not produce any significant elevation of blood pressure (Fig. 8).

Table VIII : Effect on rat blood pressure of various test substances given before $CdCl_2$

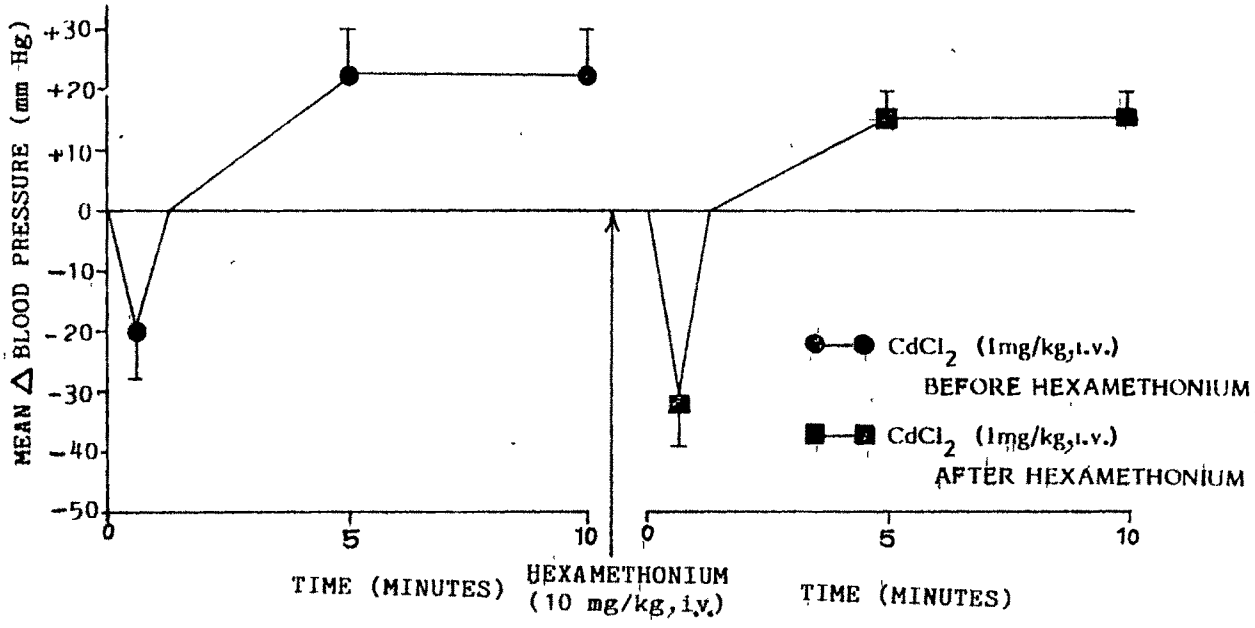
No.	Test substances	Dose mg/kg	Route	Change in B.P. (mm Hg±SEM)
1.	Phentolamine	5	i.v.	-15±3
2.	Hexamethonium	10	i.v.	-20±4
3.	Propranolol	2	i.v.	-20±3
4.	Atropine	1	i.v.	- 5±1
5.	Indomethacin	20	i.p.	-15±2
6.	Verapamil	0.5	i.v.	-12±4
		1		-20±5
		2		-30±4
7.	Nifedipine	0.25	i.v.	-15±3
		0.5		-25±4
8.	Vehicle of Nifedipine (PEG-400 15: Ethanol 15: Saline 70)	0.2 ml	i.v.	+15±2

Fig. 6 : Mean change in blood pressure (mm Hg) by acute intravenous injection of $CdCl_2$ (1 mg/kg) in anaesthetized rats depicted in all panels except D which depicts effect of intravenous tyramine (TYR 100 µg/kg). Left hand panels represent controls and right hand panels the effects of antagonists on the mean blood pressure change. The antagonists were phentolamine (5 mg/kg, i.v.), hexamethonium (10 mg/kg, i.v.), reserpine (5 mg/kg, i.p.), propranolol (2 mg/kg, i.v.), atropine (1 mg/kg, i.v.), indomethacin (20 mg/kg, i.p.), vehicle for indomethacin (0.2 ml, i.p.), verapamil (0.5 mg/kg, i.v.), verapamil (1 mg/kv, i.v.), verapamil (2 mg/kg, i.v.), nifedipine (0.25 mg/kg, i.v.), nifedipine (0.5 mg/kg, i.v.) and vehicle for nifedipine (0.2 ml, i.v.), in A, B, C, , E, F, G, H, I, J, K, L, M and N, respectively. Abscissa indicates time in min and ordinate the mean change in blood pressure (mm Hg). Vertical lines represent SEM (n=5 to 6 for each observations. * $P < 0.05$ and ** $P < 0.01$ as compared with the corresponding control).

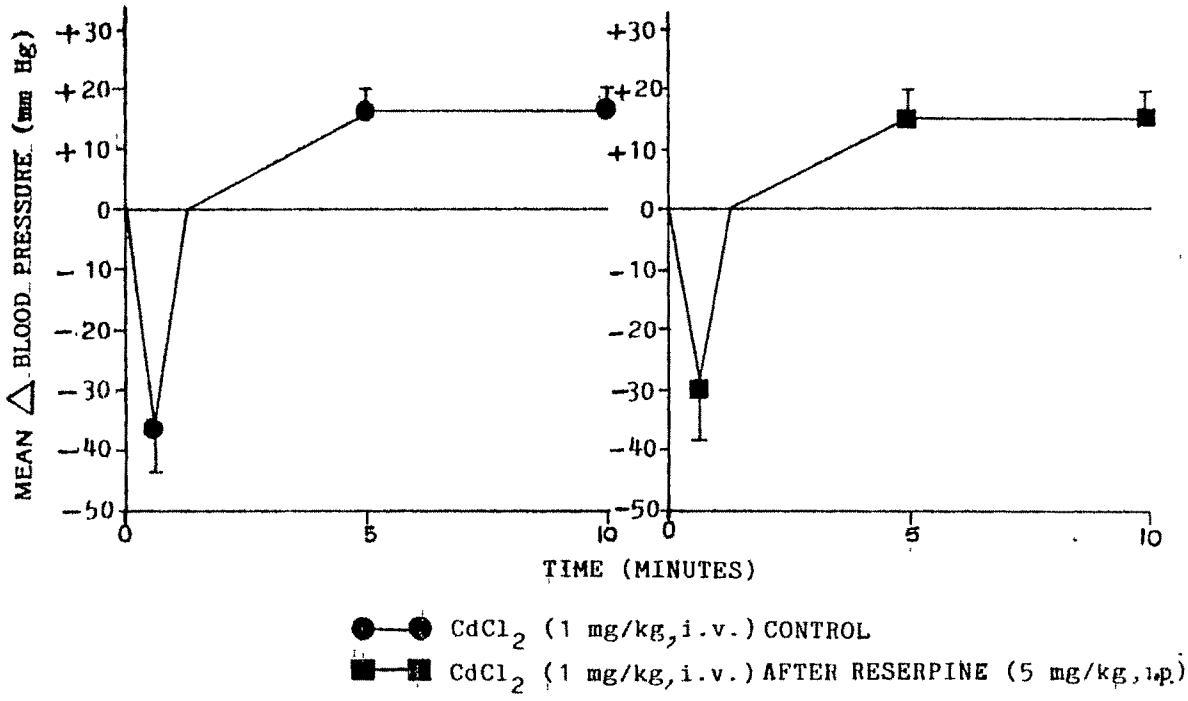
A


MEAN Δ BLOOD PRESSURE (mm-Hg)
+30
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
TIME (MINUTES)
PHENTOLAMINE
(5 mg/kg, i.v.)
CdCl2 (1mg/kg, i.v.)
BEFORE PHENTOLAMINE
CdCl2 (1mg/kg, i.v.)
AFTER PHENTOLAMINE

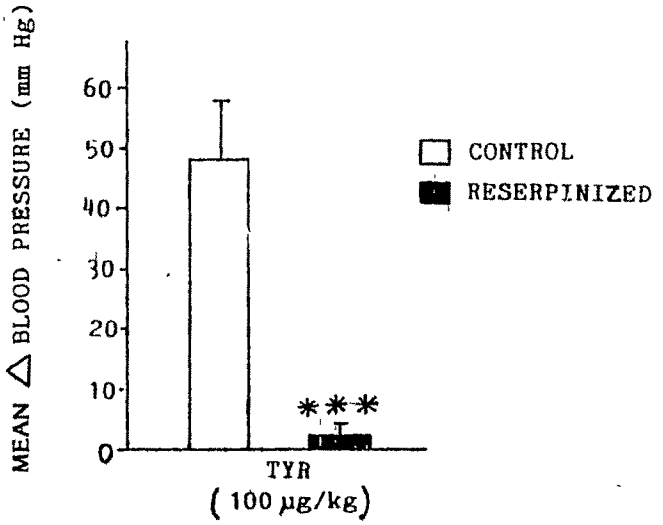
B

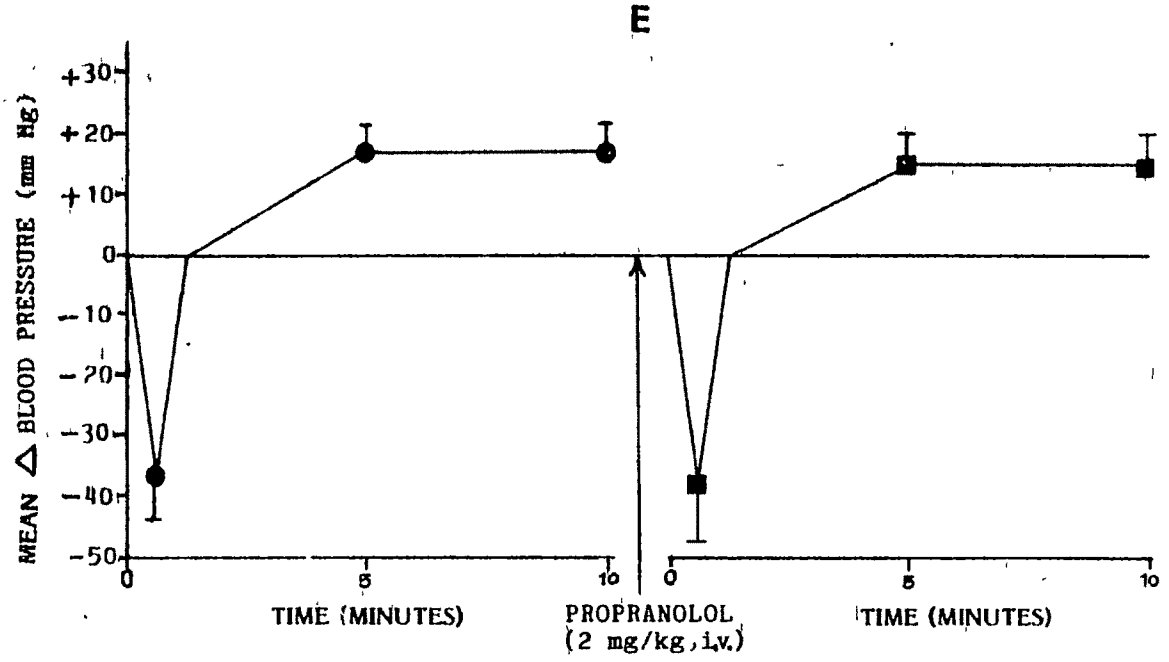

MEAN Δ BLOOD PRESSURE (mm-Hg)
+30
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
TIME (MINUTES)
HEXAMETHONIUM
(10 mg/kg, i.v.)
CdCl2 (1mg/kg, i.v.)
BEFORE HEXAMETHONIUM
CdCl2 (1mg/kg, i.v.)
AFTER HEXAMETHONIUM

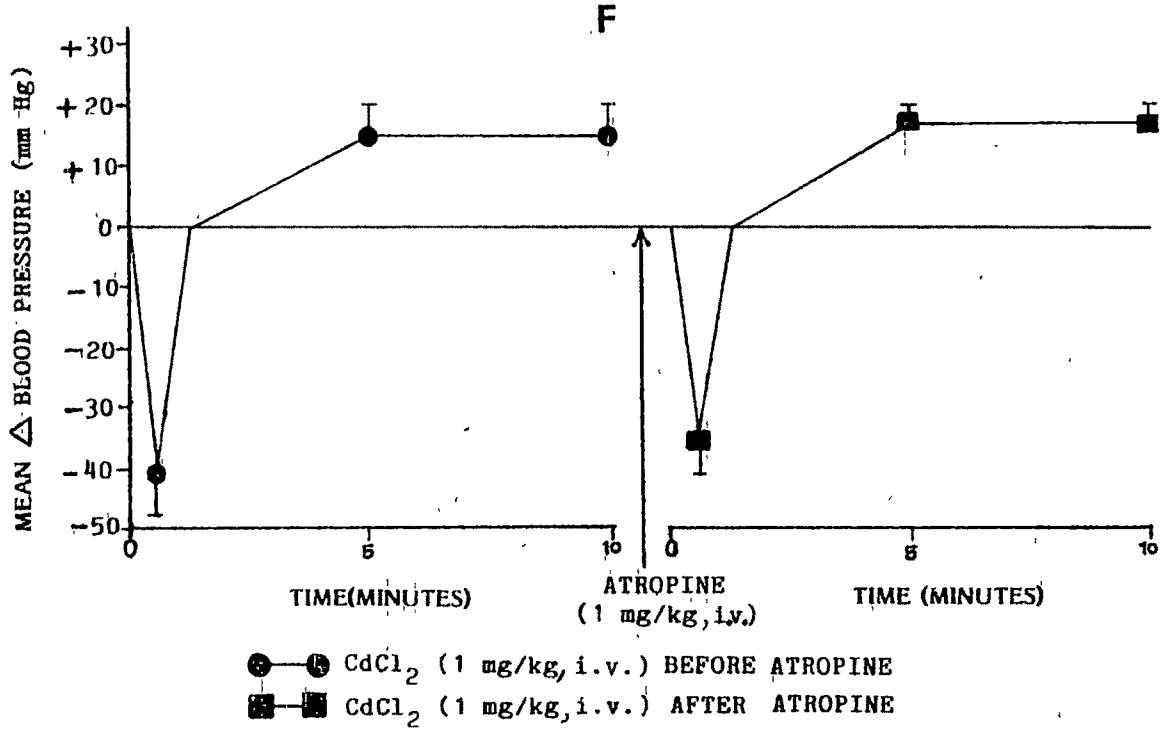
C


MEAN △ BLOOD PRESSURE (mm Hg)
+30
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
TIME (MINUTES)
CdCl2 (1 mg/kg, i.v.) CONTROL
CdCl2 (1 mg/kg, i.v.) AFTER RESERPINE (5 mg/kg, i.p.)

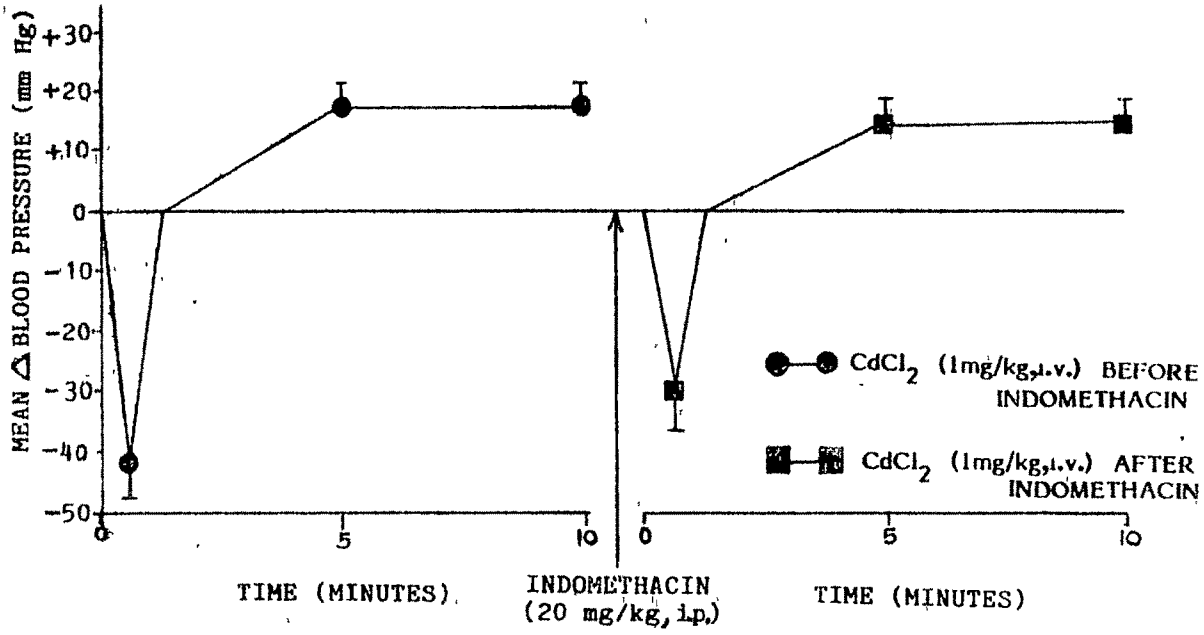
D


MEAN △ BLOOD PRESSURE (mm Hg)
60
50
40
30
20
10
0
***
TYR
(100 µg/kg)
CONTROL
RESERPINIZED

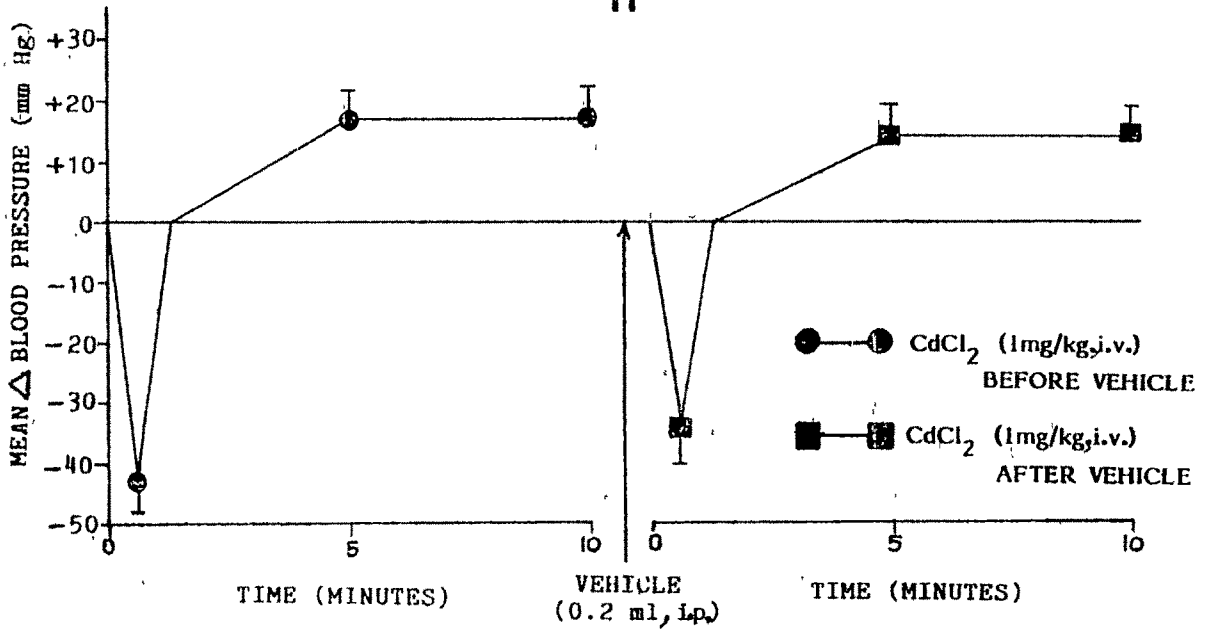

E
MEAN Δ BLOOD PRESSURE (mm Hg)
+30
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
TIME (MINUTES)
PROPRANOLOL
(2 mg/kg, i.v.)
TIME (MINUTES)
CdCl2 (1 mg/kg, i.v.) BEFORE PROPRANOLOL
CdCl2 (1 mg/kg, i.v.) AFTER PROPRANOLOL

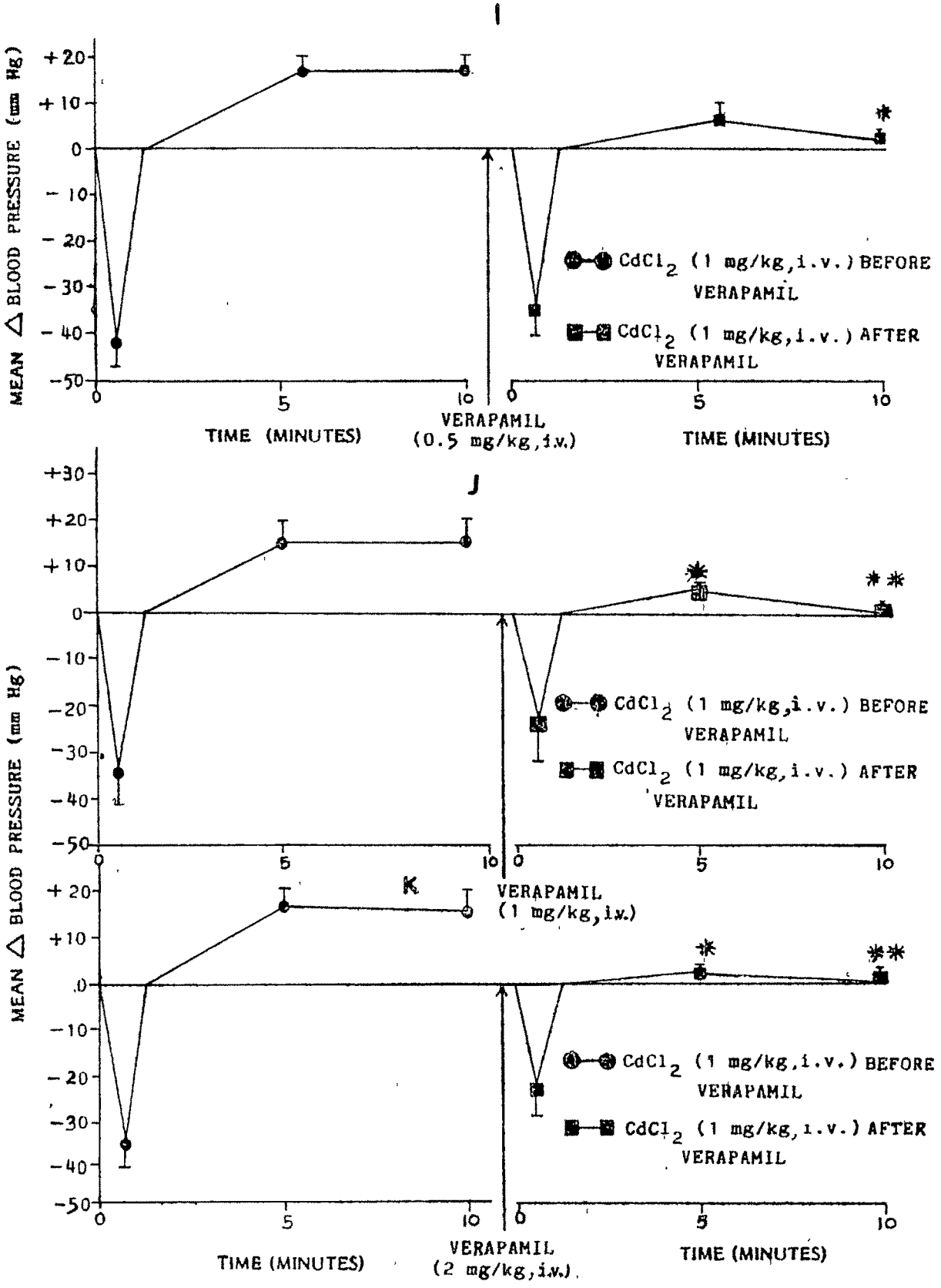

F
MEAN Δ BLOOD PRESSURE (mm Hg)
+30
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
TIME(MINUTES)
ATROPINE
(1 mg/kg, i.v.)
TIME (MINUTES)
CdCl2 (1 mg/kg, i.v.) BEFORE ATROPINE
CdCl2 (1 mg/kg, i.v.) AFTER ATROPINE

G


MEAN Δ BLOOD PRESSURE (mm Hg)
+30
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
CdCl2 (1mg/kg,i.v.) BEFORE INDOMETHACIN
CdCl2 (1mg/kg,i.v.) AFTER INDOMETHACIN
TIME (MINUTES)
INDOMETHACIN (20 mg/kg, i.p.)
TIME (MINUTES)

H


MEAN Δ BLOOD PRESSURE (mm Hg)
+30
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
CdCl2 (1mg/kg,i.v.) BEFORE VEHICLE
CdCl2 (1mg/kg,i.v.) AFTER VEHICLE
TIME (MINUTES)
VEHICLE (0.2 ml, i.p.)
TIME (MINUTES)


I
MEAN Δ BLOOD PRESSURE (mm Hg)
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
TIME (MINUTES)
VERAPAMIL
(0.5 mg/kg, i.v.)
$CdCl_2$ (1 mg/kg, i.v.) BEFORE VERAPAMIL
$CdCl_2$ (1 mg/kg, i.v.) AFTER VERAPAMIL
J
+30
+20
+10
0
-10
-20
-30
-40
-50
VERAPAMIL
(1 mg/kg, i.v.)
$CdCl_2$ (1 mg/kg, i.v.) BEFORE VERAPAMIL
$CdCl_2$ (1 mg/kg, i.v.) AFTER VERAPAMIL
K
+20
+10
0
-10
-20
-30
-40
-50
TIME (MINUTES)
VERAPAMIL
(2 mg/kg, i.v.)
TIME (MINUTES)
$CdCl_2$ (1 mg/kg, i.v.) BEFORE VERAPAMIL
$CdCl_2$ (1 mg/kg, i.v.) AFTER VERAPAMIL

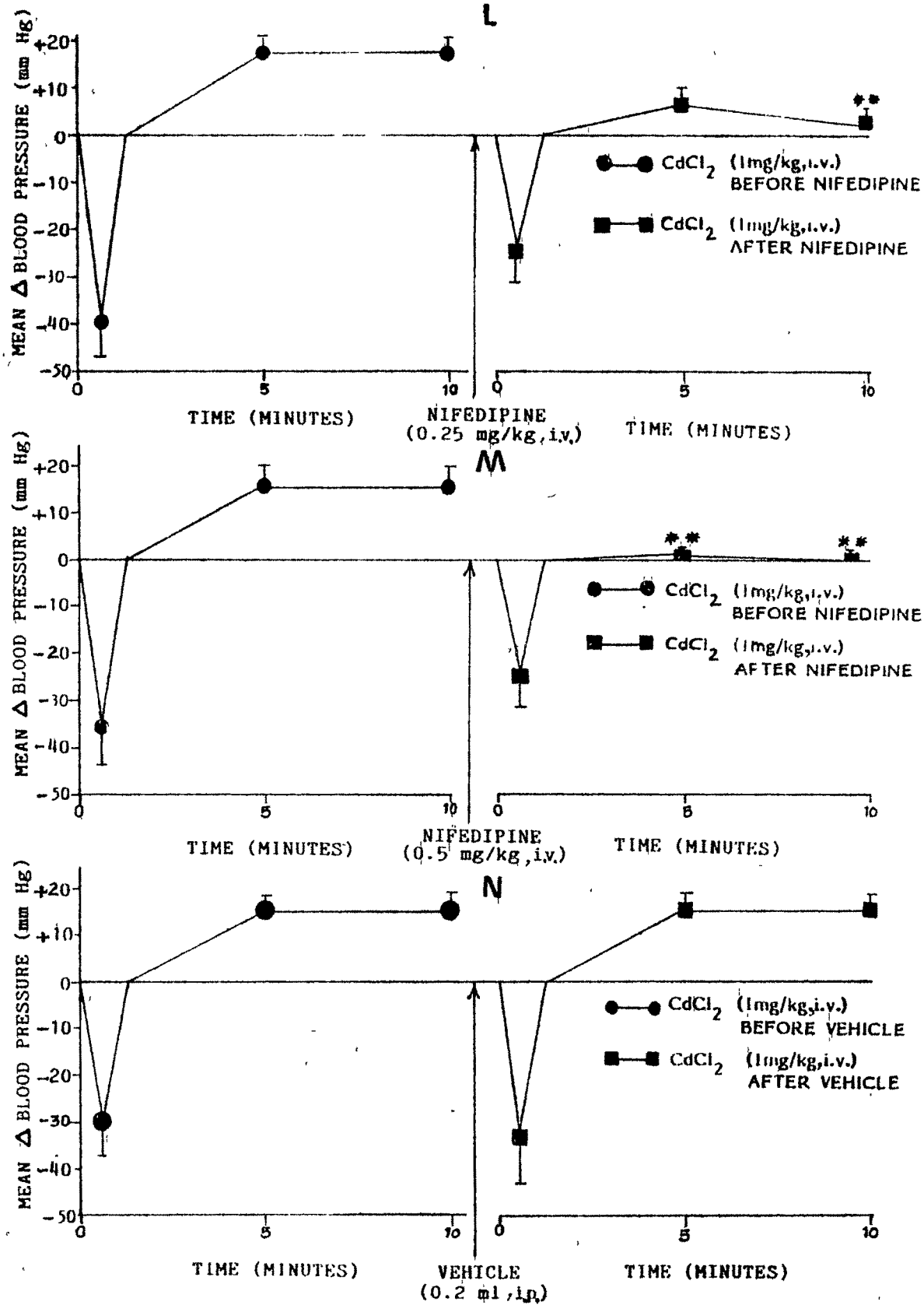
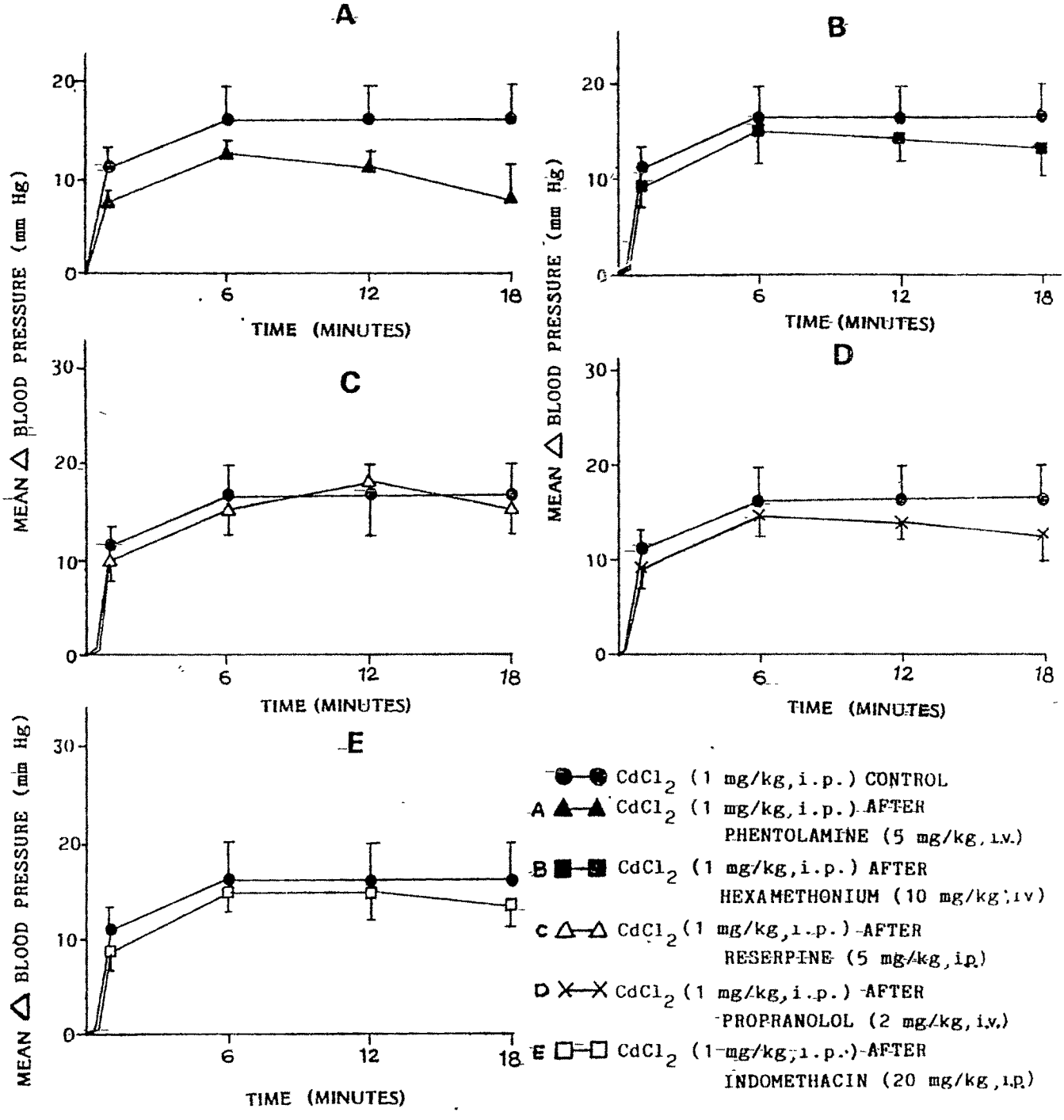
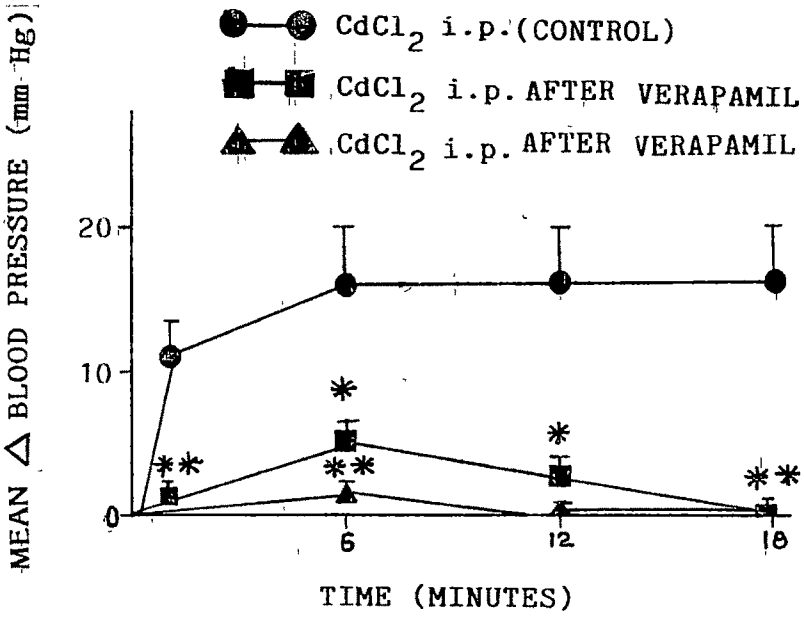

L
MEAN Δ BLOOD PRESSURE (mm Hg)
+20
+10
0
-10
-20
-30
-40
-50
0
5
10
TIME (MINUTES)
NIFEDIPINE
(0.25 mg/kg, i.v.)
TIME (MINUTES)
CdCl2 (1mg/kg, i.v.)
BEFORE NIFEDIPINE
CdCl2 (1mg/kg, i.v.)
AFTER NIFEDIPINE
**
M
MEAN Δ BLOOD PRESSURE (mm Hg)
TIME (MINUTES)
NIFEDIPINE
(0.5 mg/kg, i.v.)
TIME (MINUTES)
CdCl2 (1mg/kg, i.v.)
BEFORE NIFEDIPINE
CdCl2 (1mg/kg, i.v.)
AFTER NIFEDIPINE
**
N
MEAN Δ BLOOD PRESSURE (mm Hg)
TIME (MINUTES)
VEHICLE
(0.2 ml, i.p.)
TIME (MINUTES)
CdCl2 (1mg/kg, i.v.)
BEFORE VEHICLE
CdCl2 (1mg/kg, i.v.)
AFTER VEHICLE

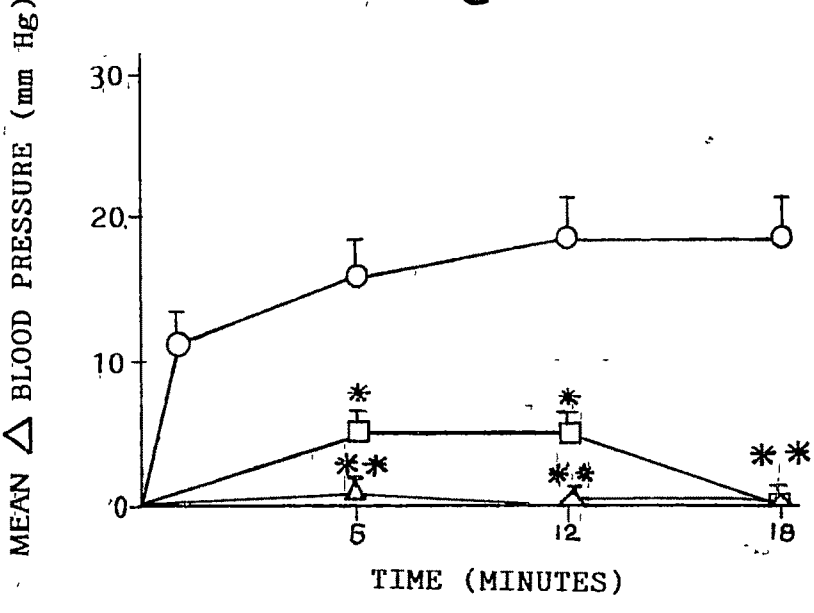
Fig. 7 : Mean change in blood pressure produced by acute intraperitoneal injection of $CdCl_2$ (1 mg/kg) in anaesthetized rats in control and after various antagonists. The antagonists were phentolamine (5 mg/kg, i.v.), hexamethonium (10 mg/kg, i.v.), reserpine (5 mg/kg, i.p.), propranolol (2 mg/kg, i.v.) indomethacin (20 mg/kg, i.p.), verapamil (0.5 and 1 mg/kg, i.v.), nifedipine (0.25 and 5 mg/kg i.v.) and vehicle for nifedipine in A,B,C,D,E,F, and G respectively. Abscissa indicates the time in min and ordinate the mean change in blood pressure (mm Hg). Vertical lines represent SEM (n=5 to 6 for each observation. * $P < 0.05$ and ** $P < 0.01$ as compared with corresponding control).


A
B
C
D
E
MEAN Δ BLOOD PRESSURE (mm Hg)
TIME (MINUTES)
0
10
20
30
6
12
18
$CdCl_2$ (1 mg/kg, i.p.) CONTROL
A $CdCl_2$ (1 mg/kg, i.p.) AFTER PHENTOLAMINE (5 mg/kg, i.v.)
B $CdCl_2$ (1 mg/kg, i.p.) AFTER HEXAMETHONIUM (10 mg/kg, i.v.)
C $CdCl_2$ (1 mg/kg, i.p.) AFTER RESERPINE (5 mg/kg, i.p.)
D $CdCl_2$ (1 mg/kg, i.p.) AFTER PROPRANOLOL (2 mg/kg, i.v.)
E $CdCl_2$ (1 mg/kg, i.p.) AFTER INDOMETHACIN (20 mg/kg, i.p.)

F



G



CdCl2 i.p. AFTER VEHICLE

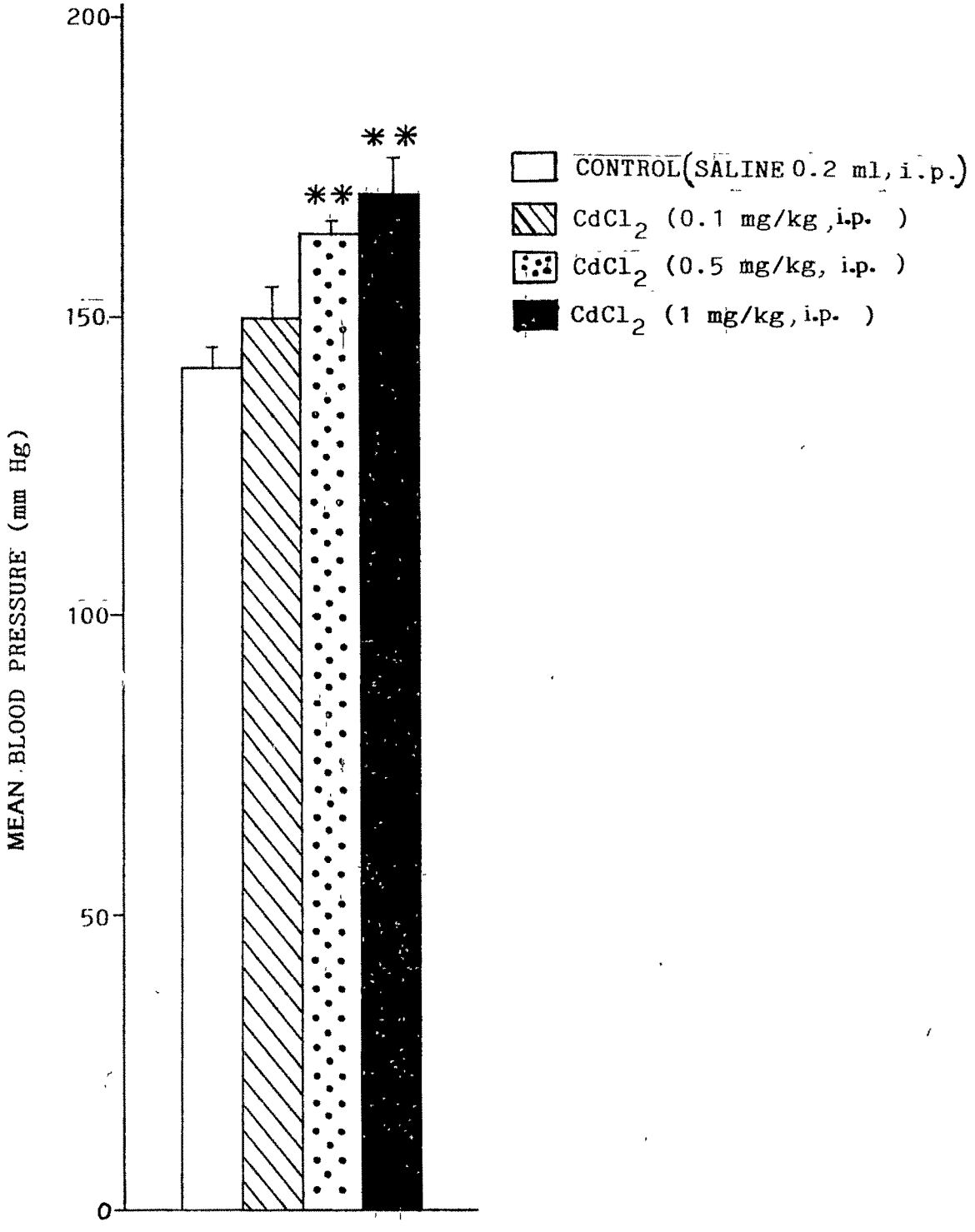
CdCl2 i.p. AFTER NIFEDIPINE (0.25 mg/kg,i.v.)

CdCl2 i.p. AFTER NIFEDIPINE (0.5 mg/kg,i.v.)

Table IX : Effect on systolic blood pressure (mm Hg) of female rats exposed to different concentrations of $CdCl_2$ in deionized water (n=6)

Group	Duration of exposure	Systolic blood pressure (mm Hg±SEM)				P
		$CdCl_2$ concentration (ppm)				
		Control	5	25	100	
A	4 weeks	115±7	125±10	122±8	101±7	>0.05
B	8 weeks	110±11	117±4	125±5	119±5	>0.05

Fig. 8 : Mean blood pressure (mm Hg) of groups of female rats. White histogram indicates control experiments (0.2 ml saline, i.p./day for 2 weeks), hatched, stippled and black histograms indicate chronic treatment with 0.1 mg/kg/day, i.p., 0.5 mg/kg/day i.p. and 1 mg/kg/day, i.p. dose of $CdCl_2$ for two weeks respectively. Vertical lines on histograms represent SEM (n=20 for control and for 1 mg/kg group; and 6 for others.
** $P < 0.01$ as compared with corresponding control).


200
150
100
50
0
MEAN BLOOD PRESSURE (mm Hg)
**
**
CONTROL(SALINE 0.2 ml, i.p.)
CdCl2 (0.1 mg/kg, i.p.)
CdCl2 (0.5 mg/kg, i.p.)
CdCl2 (1 mg/kg, i.p.)

4.1.2.2. VASCULAR REACTIVITY TO AGONISTS IN TREATED ANIMALS:

The blood pressure responses to different doses of NA (0.5, 1 and 2 μg/kg) were not modified in animals chronically treated with higher doses of $CdCl_2$ (0.5 and 1 mg/kg, i.p.). However, in animals treated with the lower dose (0.1 mg/kg, i.p.), the blood pressure responses to NA (1 and 2 μg/kg) were significantly potentiated ($P < 0.05$)(Fig. 9A). Responses to ANG II, isoprenaline and ACh were not affected by any of the doses studied (Fig. 9B and 10).

4.1.2.3. EXPERIMENTS TO EVALUATE THE MECHANISM OF HYPERTENSION DUE TO CHRONIC $CdCl_2$:

4.1.2.3.1. Effect of adrenalectomy on $CdCl_2$ induced hypertension :

In sham operated rats, chronic $CdCl_2$ (1 mg/kg, i.p.) treatment produced ($P < 0.01$) elevation of blood pressure. Bilateral adrenalectomy produced significant ($P < 0.05$) lowering of the blood pressure of untreated animals. Bilateral adrenalectomy did not prevent the development of hypertension produced by chronic $CdCl_2$ treatment; the blood pressure of $CdCl_2$-treated bilaterally adrenalectomized animals was not significantly different from $CdCl_2$-treated sham operated animals (Fig. 11). The acute responses to intravenous (Fig. 12A) or intraperitoneal (Fig. 12B)

Fig. 9 : Mean change in blood pressure (mm Hg) produced by intravenous injection of NA (0.5, 1 and 2 μg/kg, A) and ANG II (50, 100 and 200 ng/kg, B) in anaesthetized rats. ●—● indicates control and (■—■), (▲—▲) and (×—×) indicate 0.1 mg/kg, i.p., 0.5 mg/kg i.p. and 1 mg/kg i.p. doses of $CdCl_2$ administered chronically for two weeks respectively. Vertical lines represent the SEM (n=5 to 6 for each observation; * $P < 0.05$ as compared with corresponding control).

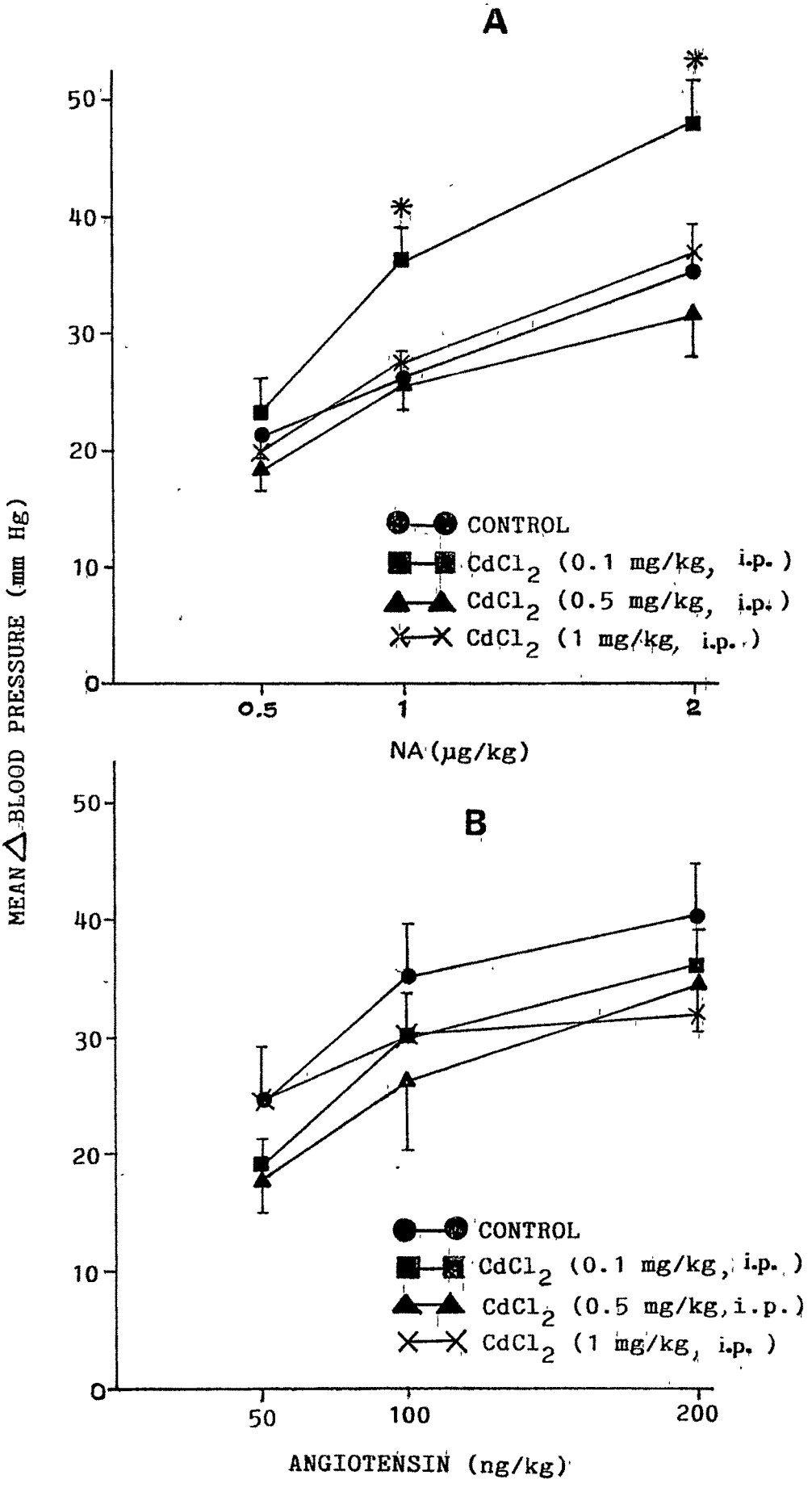

A
50
40
30
20
10
0
0.5
1
2
NA (μg/kg)
CONTROL
CdCl2 (0.1 mg/kg, i.p.)
CdCl2 (0.5 mg/kg, i.p.)
CdCl2 (1 mg/kg, i.p.)
B
50
40
30
20
10
0
50
100
200
ANGIOTENSIN (ng/kg)
CONTROL
CdCl2 (0.1 mg/kg, i.p.)
CdCl2 (0.5 mg/kg, i.p.)
CdCl2 (1 mg/kg, i.p.)
MEAN Δ BLOOD PRESSURE (mm Hg)

Fig. 10 : Mean change in blood pressure (mm Hg) produced by the administration of intravenous injection of isoprenaline (0.5 and 1 μg/kg, A) and ACh (50 and 100 ng/kg, B) in anaesthetized rats. White histogram indicates control and black, diagonally hatched, vertically hatched histograms indicate 0.1 mg/kg, i.p., 0.5 mg/kg, i.p., and 1 mg/kg, i.p., doses of $CdCl_2$ administered chronically for two weeks respectively. Vertical lines on histograms represent SEM (n=4 to 5 for each observation).

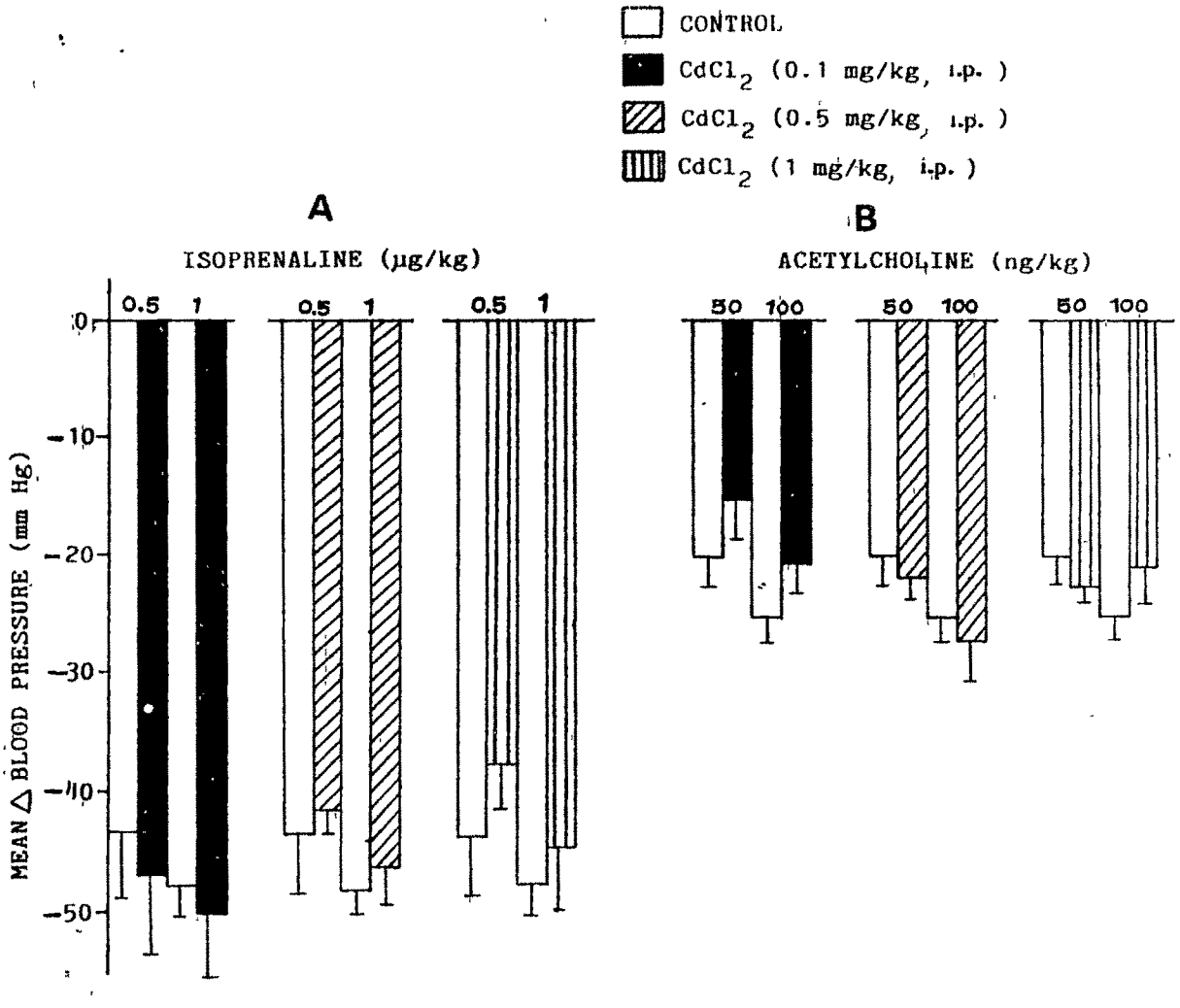

CONTROL
CdCl2 (0.1 mg/kg, i.p.)
CdCl2 (0.5 mg/kg, i.p.)
CdCl2 (1 mg/kg, i.p.)
A
ISOPRENALINE (µg/kg)
B
ACETYLCHOLINE (ng/kg)
MEAN Δ BLOOD PRESSURE (mm Hg)
0
-10
-20
-30
-40
-50
0.5
1
50
100

Fig. 11 : Mean blood pressure (mm Hg) of groups of anaesthetized female rats. (a) White histogram indicates sham operated control (0.2 ml saline/day, i.p., for two weeks), (b) stippled (c) black and (d) hatched histograms indicate chronic $CdCl_2$ (1 mg/kg/day, i.p. for two weeks) injected in sham operated, adrenalectomized and $CdCl_2$ injected in adrenalectomized animals respectively. Vertical lines on histograms represent SEM (n=5 to 8 for each observation. a vs c = * $P < 0.05$, a vs b = P ** < 0.01)

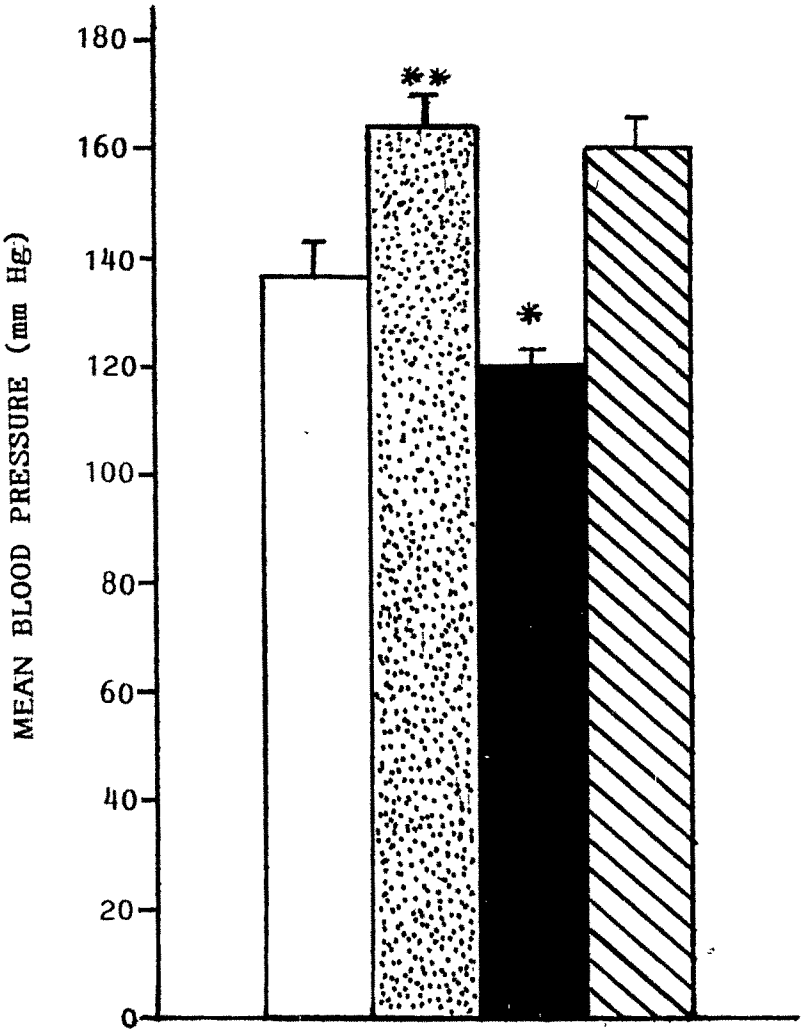
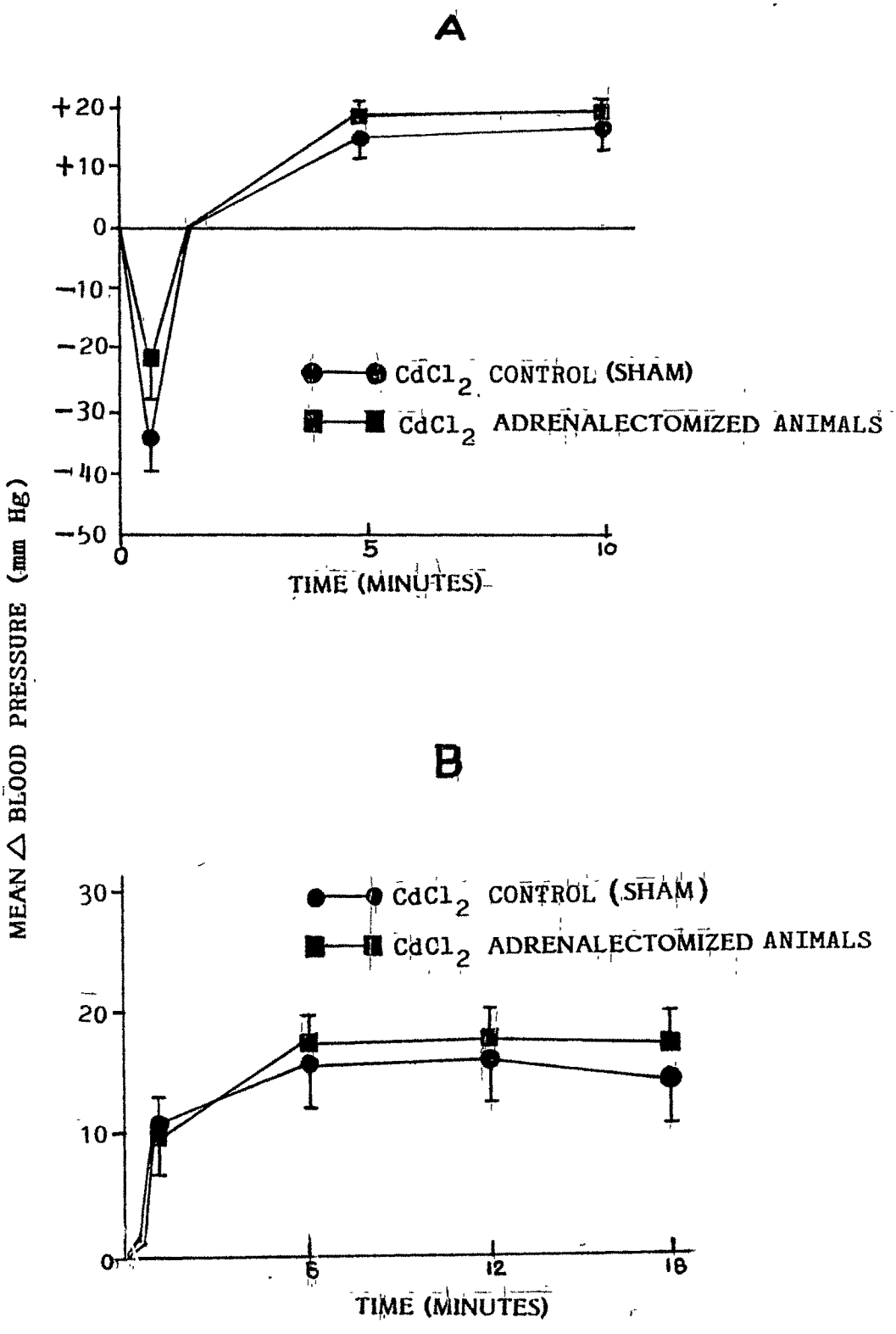

MEAN BLOOD PRESSURE (mm Hg)
180
160
140
120
100
80
60
40
20
0
**
*
(a) CONTROL (SHAM)
(b) SHAM + $CdCl_2$
(c) ADRENALECTOMIZED
(d) ADRENALECTOMIZED + $CdCl_2$

Fig. 12 : Mean change in blood pressure produced by acute intravenous (A) and intraperitoneal (B) injections of $CdCl_2$ (1 mg/kg) in sham operated control and in adrenalectomized anaesthetized rats. Abscissa indicates time in min and ordinate the mean change in blood pressure (mm Hg). Vertical lines represent SEM (n=4 for each observation).


A
+20
+10
0
−10
−20
−30
−40
−50
0
5
10
TIME (MINUTES)
$CdCl_2$ CONTROL (SHAM)
$CdCl_2$ ADRENALECTOMIZED ANIMALS
MEAN Δ BLOOD PRESSURE (mm Hg)
B
30
20
10
0
6
12
18
TIME (MINUTES)
$CdCl_2$ CONTROL (SHAM)
$CdCl_2$ ADRENALECTOMIZED ANIMALS

administration of $CdCl_2$ (1 mg/kg) were not modified in adrenalectomized animals.

4.1.2.3.2. Effect of chemical sympathectomy on $CdCl_2$-induced hypertension :

Chemical sympathectomy by guanethidine lowered ($P < 0.05$) the basal blood pressure of untreated animals, however, chemical sympathectomy did not prevent the hypertension induced by chronic $CdCl_2$ treatment; the blood pressure of chemically sympathectomized-$CdCl_2$ treated animals was not significantly different from that of $CdCl_2$ treated animals (Fig. 13). When TYR (100 µg/kg) was given intravenously in guanethidine treated animals, there was no pressor response ($P < 0.001$) confirming complete sympathectomy. Furthermore, there was significant potentiation of NA (1 µg/kg, i.v.) pressor response ($P < 0.01$) in chemically sympathectomized animals (Fig. 14). The acute pressor responses to intravenous (Fig. 15A) or intraperitoneal (Fig. 15B) administration of $CdCl_2$ (1 mg/kg) were not modified in guanethidine treated animals.

4.1.2.3.3. Effect of captopril on $CdCl_2$ induced hypertension :

Captopril treatment lowered ($P < 0.05$) the blood pressure of the untreated group. However, the drug did not prevent the hypertension-induced by chronic $CdCl_2$ administration; the

Fig. 13 : Mean blood pressure (mm Hg) of groups of anaesthetized female rats. (a) White histogram indicates control (0.2 ml saline/day, i.p., for two weeks), (b) stippled (c) black and (d) hatched histograms indicate chronic $CdCl_2$ treated (1 mg/kg/day, i.p., for two weeks), sympathectomized (guanethidine 50 mg/kg/day, i.p., for 5 weeks) and $CdCl_2$ + sympathectomized animals respectively. Vertical lines on histograms represent SEM (n=5 to 8 for each observation; a vs c = * $P < 0.05$, a vs b =** $P < 0.01$).

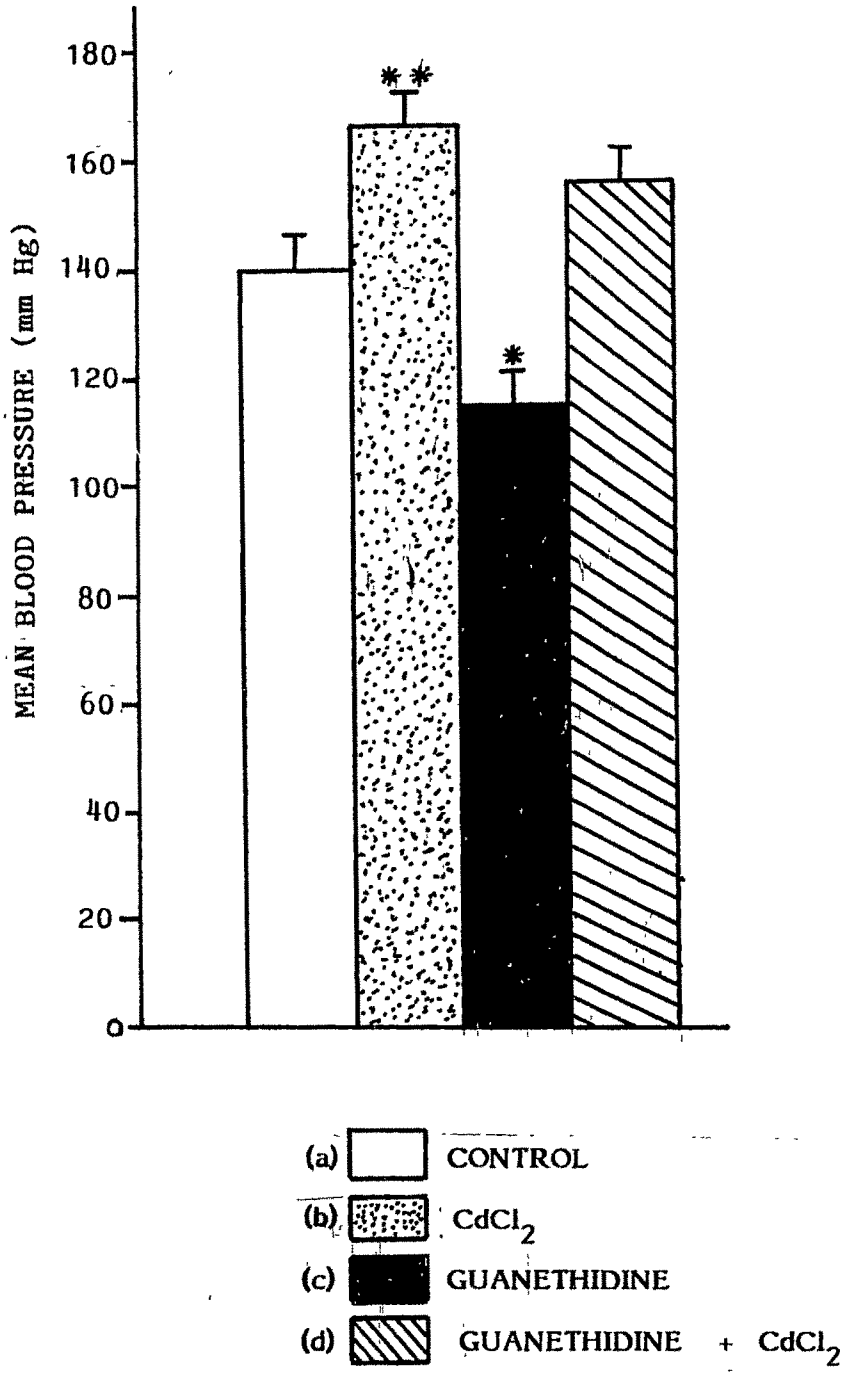

MEAN BLOOD PRESSURE (mm Hg)
180
160
140
120
100
80
60
40
20
0
**
*
(a) CONTROL
(b) CdCl2
(c) GUANETHIDINE
(d) GUANETHIDINE + CdCl2

Fig. 14 : Mean change in blood pressure (mm Hg) produced by the acute intravenous administration of TYR (100 μg/kg) and NA (1 μg/kg) in control and in guanethidine treated anaesthetized rats. Vertical lines represent SEM (n=4 to 5 for each observation. ** $P < 0.01$ and *** $P < 0.001$).

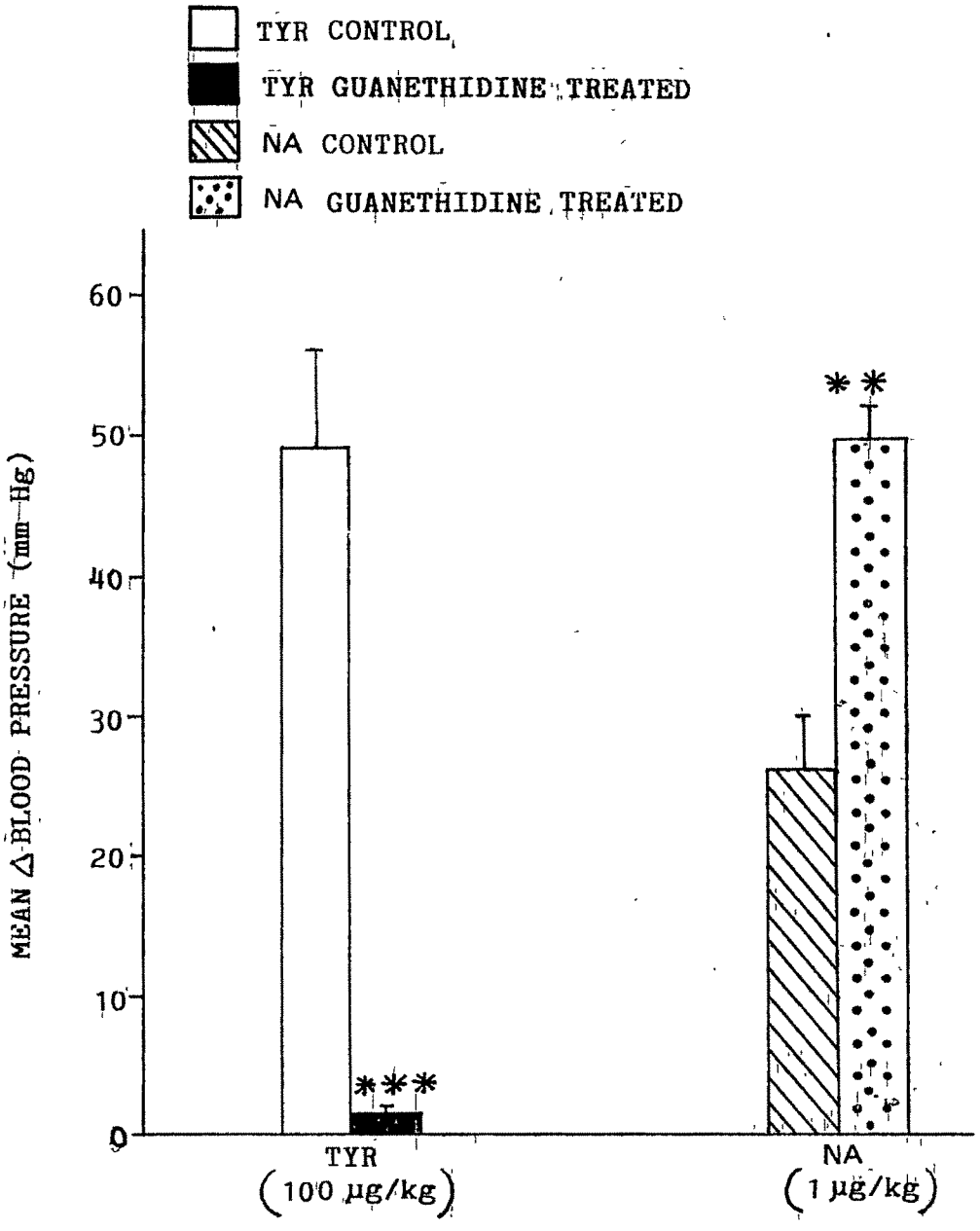
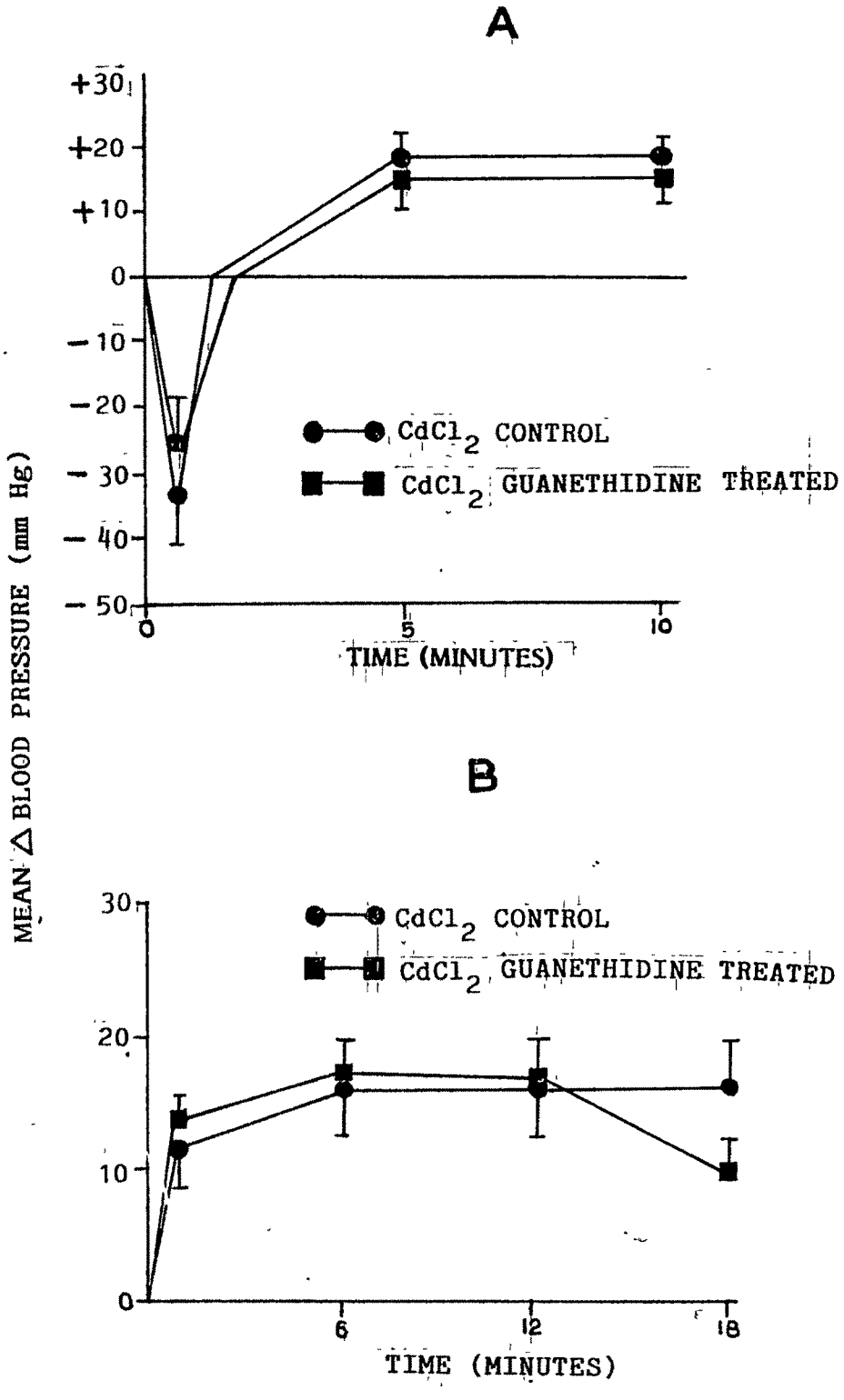

TYR CONTROL
TYR GUANETHIDINE TREATED
NA CONTROL
NA GUANETHIDINE TREATED
MEAN Δ BLOOD PRESSURE (mm-Hg)
60
50
40
30
20
10
0
***
**
TYR
(100 µg/kg)
NA
(1 µg/kg)

Fig. 15 : Mean change in blood pressure (mm Hg) by the acute intravenous (A) and intraperitoneal (B) injection of $CdCl_2$ (1 mg/kg) in control and in guanethidine (50 mg/kg/day, i.p., for five weeks) treated anaesthetized rats. Abscissa indicates the time in min and ordinate the change in blood pressure. Vertical lines represent SEM (n=4 for each observation).


A
+30
+20
+10
0
−10
−20
−30
−40
−50
0
5
10
TIME (MINUTES)
MEAN Δ BLOOD PRESSURE (mm Hg)
CdCl2 CONTROL
CdCl2 GUANETHIDINE TREATED
B
30
20
10
0
6
12
18
TIME (MINUTES)
CdCl2 CONTROL
CdCl2 GUANETHIDINE TREATED

blood pressure of the captopril-$CdCl_2$ treated animals was not significantly different from $CdCl_2$ treated animals (Fig. 16). The acute pressor effects of intravenous (Fig. 17A) or intraperitoneal (Fig. 17B) administration of $CdCl_2$ (1 mg/kg) were not modified in captopril treated animals.

4.1.2.3.4. Effect of calcium channel blockers on $CdCl_2$-induced hypertension :

Verapamil (15 mg/kg, two times/day, p.o. and 30 mg/kg/day, p.o.) or nifedipine (10 mg/kg/day, p.o.) for two weeks reduced ($P < 0.05$) the blood pressure of the untreated animals and also prevented the hypertension induced by chronic $CdCl_2$ administration. The blood pressure of verapamil-$CdCl_2$ (Fig. 18) or nifedipine-$CdCl_2$ (Fig. 20) treated animals were not significantly different from the untreated groups (Verapamil alone or nifedipine alone) but significantly ($P < 0.01$) lower than the $CdCl_2$ treated group. The acute pressor responses to intravenous (Fig. 19A and 21A) or intraperitoneal (Fig. 19B and 21B) administration of $CdCl_2$ (1 mg/kg) were significantly ($P < 0.05$ and $P < 0.01$) reduced in the verapamil or nifedipine treated animals.

4.2. IN VITRO STUDIES

4.2.1. ACUTE EXPERIMENTS

4.2.1.1. RAT HINDQUARTER PREPARATION :

Acute intra-arterial administration of $CdCl_2$ (0.5, 1 and

Fig. 16 : Mean blood pressure (mm Hg) of anaesthetized female rats. (a) White histogram indicates control (0.2 ml saline/day, i.p. for two weeks), (b) stippled (c) black and (d) hatched histograms indicate chronic $CdCl_2$ (1 mg/kg/day, i.p., for two weeks), captopril (3 mg/kg/day, p.o., for two weeks) and captopril + $CdCl_2$ treated animals respectively. Vertical line on histogram represent SEM (n=5 to 8 for each observation; a vs c = * $P < 0.05$; a vs b = ** $P < 0.01$).

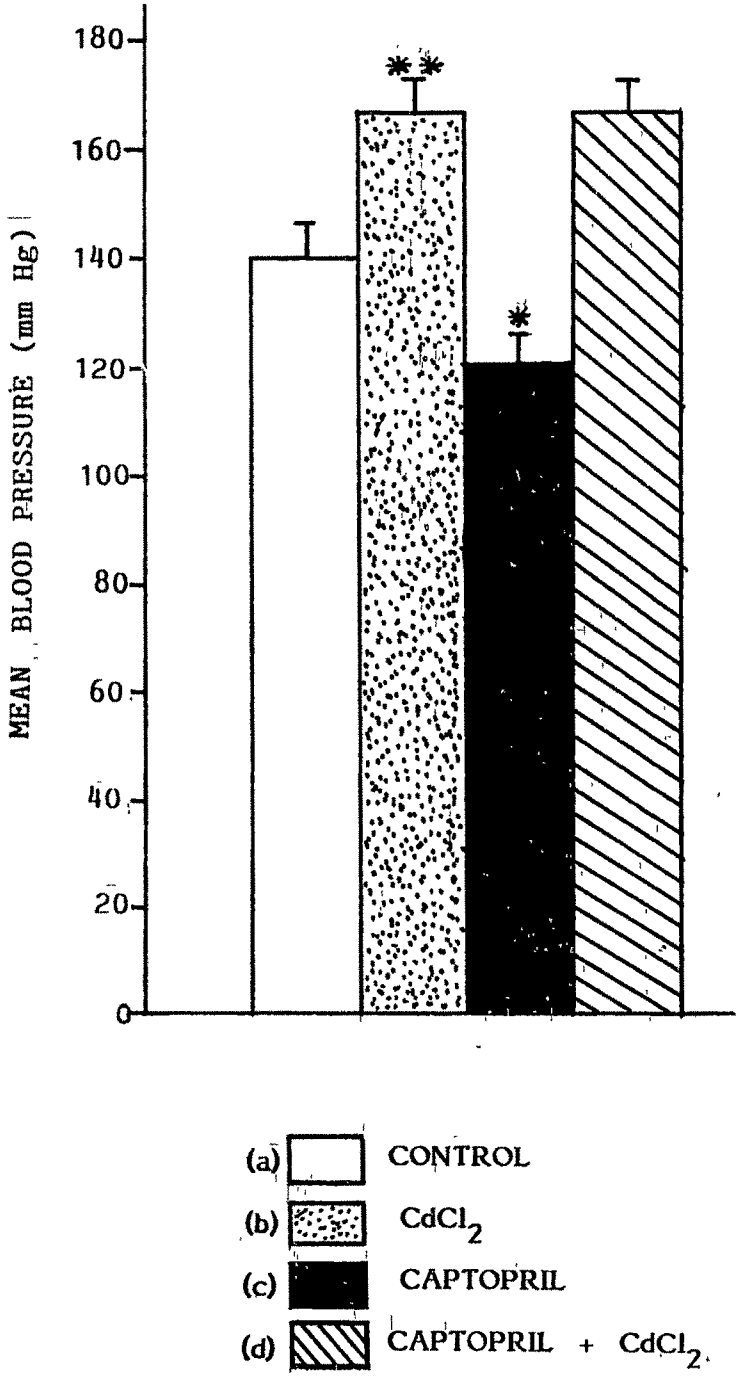

MEAN BLOOD PRESSURE (mm Hg)
180
160
140
120
100
80
60
40
20
0
(a) CONTROL
(b) $CdCl_2$
(c) CAPTOPRIL
(d) CAPTOPRIL + $CdCl_2$

Fig. 17 : Mean change in blood pressure (mm Hg) produced by the acute intravenous (A) and intraperitoneal (B) injections of $CdCl_2$ (1 mg/kg) in control and in captopril, (3 mg/kg/day, p.o., two weeks) treated anaesthetized rats. Abscissa indicates time in min and ordinate the change in blood pressure. Vertical lines represent SEM (n=4 for each observation).

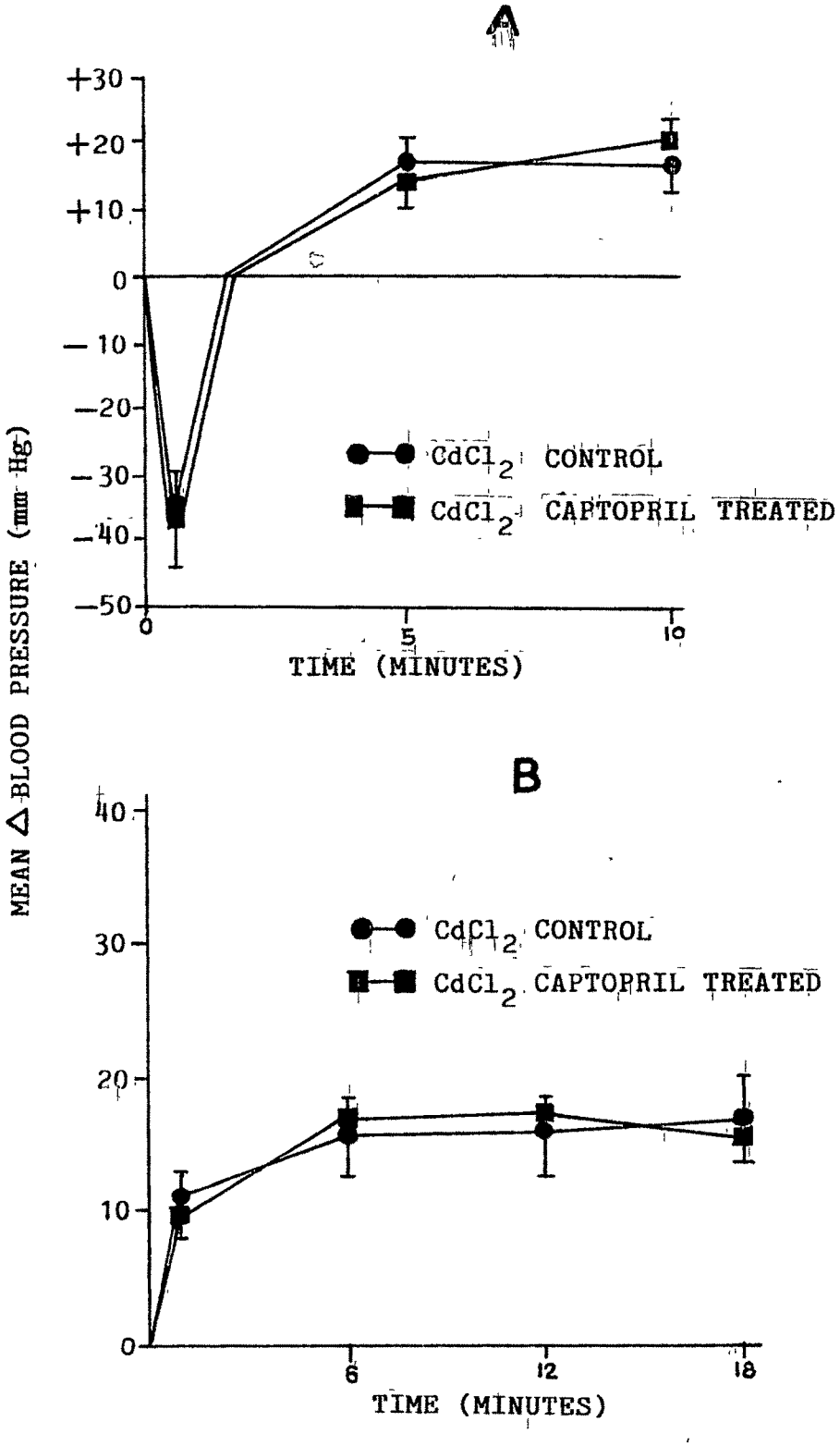

A
+30
+20
+10
0
−10
−20
−30
−40
−50
0
5
10
TIME (MINUTES)
MEAN Δ BLOOD PRESSURE (mm Hg)
$CdCl_2$ CONTROL
$CdCl_2$ CAPTOPRIL TREATED
B
40
30
20
10
0
6
12
18
TIME (MINUTES)
$CdCl_2$ CONTROL
$CdCl_2$ CAPTOPRIL TREATED

Fig. 18 : Mean blood pressure (mm Hg) of female anaesthetized rats. (a) White histogram indicates control (0.2 ml/day saline, i.p., for two weeks) (b) Stippled (c) black (d) diagonally hatched (e) circled and (f) vertically hatched histograms indicate chronic $CdCl_2$ (1 mg/kg/day, i.p., for two weeks), verapamil (15 mg/kg two times daily, p.o., for two weeks), verapamil (15 mg/kg two times daily, p.o., for two weeks) + $CdCl_2$, verapamil (30 mg/kg/day, p.o., for two weeks) alone and verapamil (30 mg/kg/day, p.o., for two tweeks) + $CdCl_2$ treated animals respectively. Vertical lines on histograms represent SEM (n=5 to 8 for each observation; a vs c and e = * $P<0.05$, a vs b = ** $P<0.01$, b vs d = ** $P<0.01$, b vs f = ** $P<0.01$).

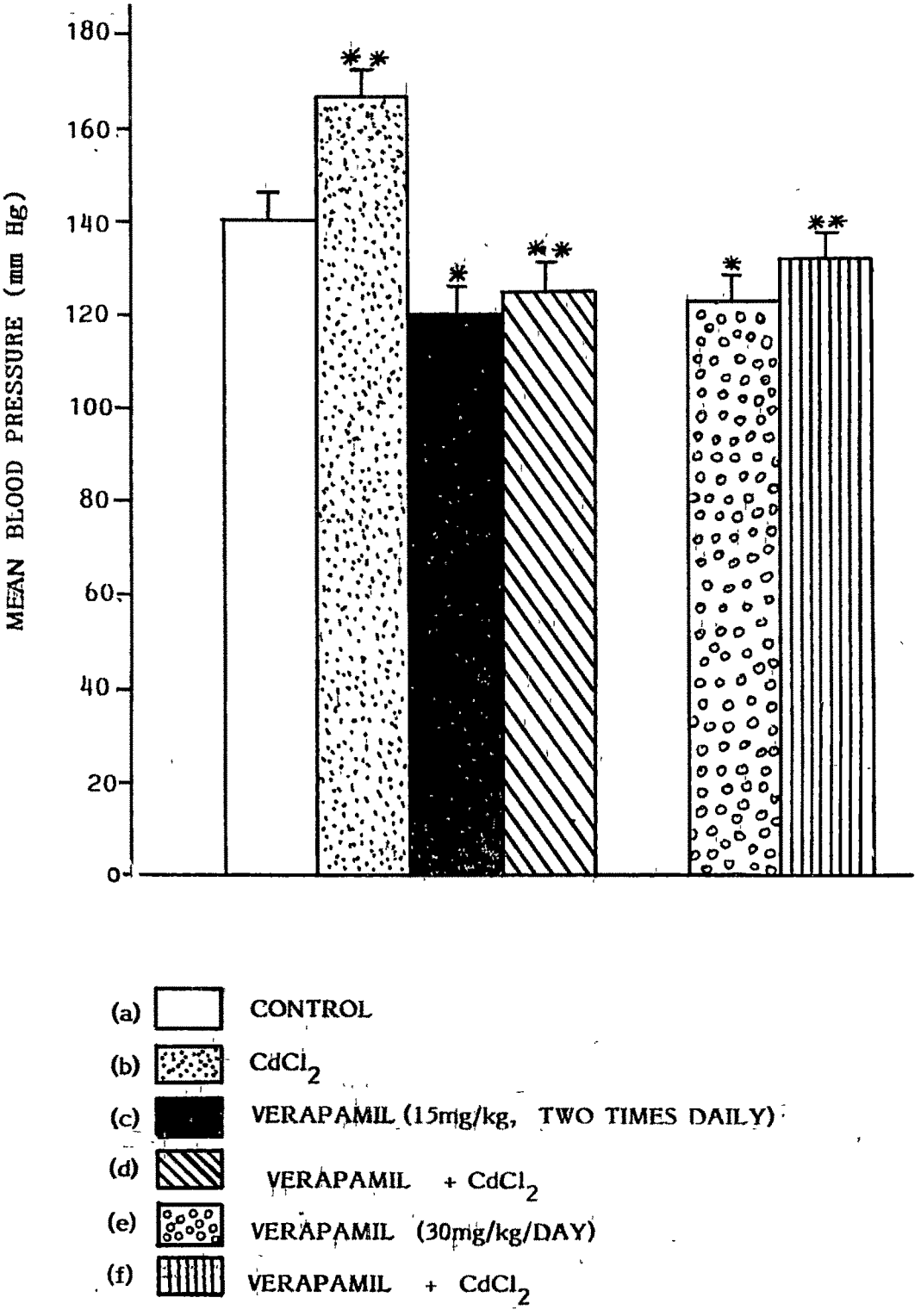

MEAN BLOOD PRESSURE (mm Hg)
180
160
140
120
100
80
60
40
20
0
(a) CONTROL
(b) $CdCl_2$
(c) VERAPAMIL (15mg/kg, TWO TIMES DAILY)
(d) VERAPAMIL + $CdCl_2$
(e) VERAPAMIL (30mg/kg/DAY)
(f) VERAPAMIL + $CdCl_2$

Fig. 19 : Mean change in blood pressure (mm Hg) produced by acute intravenous (A) and intraperitoneal (B) injections of $CdCl_2$ in control and in verapamil (15 mg/kg, two times daily, p.o., for two weeks and 30 mg/kg/day, p.o., for two weeks) treated anaesthetized rats. Abscissa indicates time in min and ordinate the change in blood pressure. Vertical lines represent SEM (n=4 for each observation; * $P < 0.05$ and ** $P < 0.01$ as compared with corresponding control.)

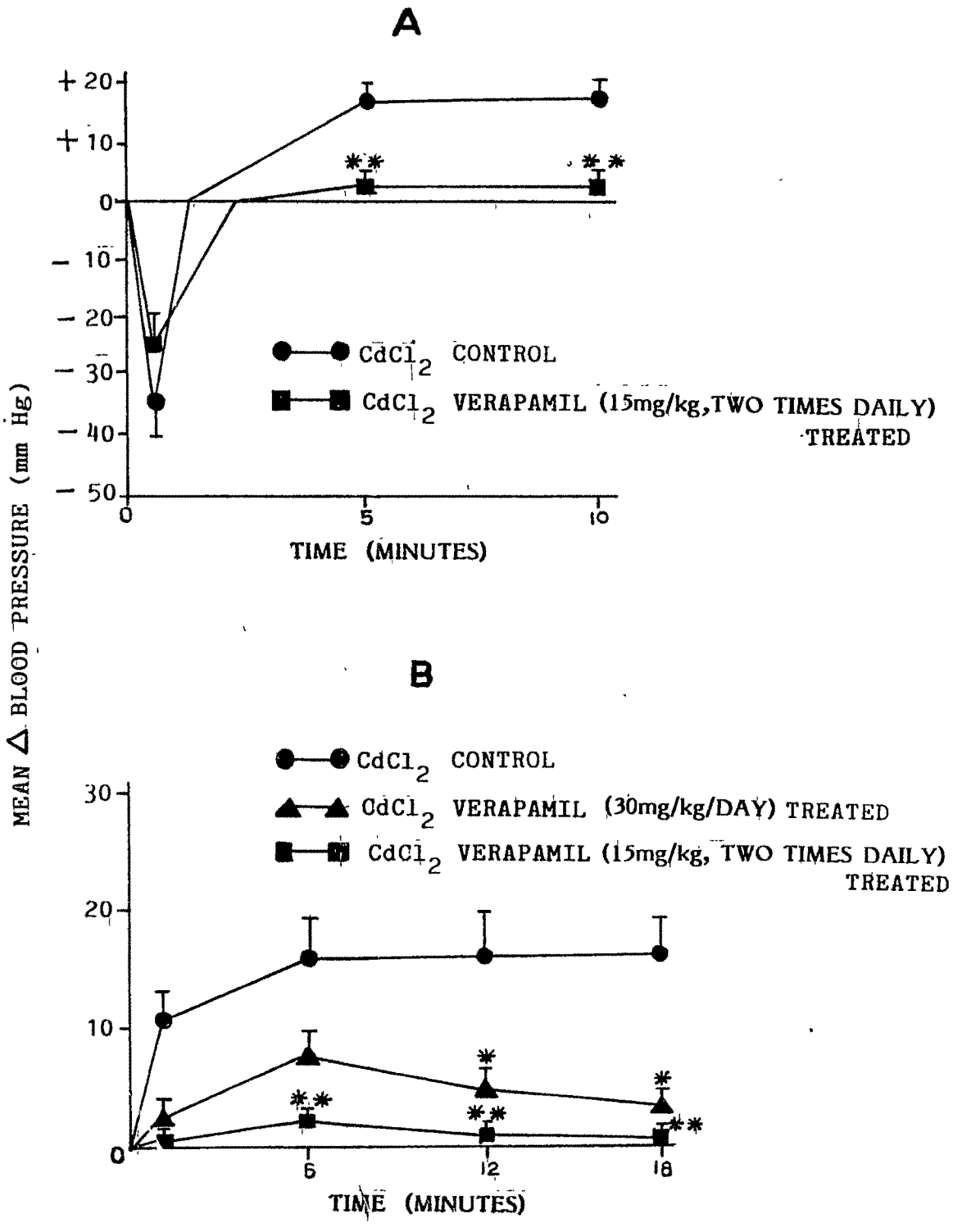

A
+20
+10
0
−10
−20
−30
−40
−50
0
5
10
TIME (MINUTES)
CdCl2 CONTROL
CdCl2 VERAPAMIL (15mg/kg,TWO TIMES DAILY) TREATED
B
30
20
10
0
6
12
18
TIME (MINUTES)
CdCl2 CONTROL
CdCl2 VERAPAMIL (30mg/kg/DAY) TREATED
CdCl2 VERAPAMIL (15mg/kg, TWO TIMES DAILY) TREATED
MEAN Δ BLOOD PRESSURE (mm Hg)

Fig. 20 : Mean blood pressure (mm Hg) of anaesthetized female rats. (a) White histogram indicates vehicle control (0.2 ml/day, PEG-400 p.o. for two weeks + 0.2 ml, saline/day i.p. for two weeks), (b) stippled, (c) black and (d) hatched histograms indicate chronic $CdCl_2$ (1 mg/kg/day, i.p. for two weeks + 0.2 ml PEG-400/day, p.o., for two weeks), nifedipine (10 mg/kg/day, p.o., for two weeks) and nifedipine + $CdCl_2$ treated animals respectively. Vertical lines on histograms represent SEM (n=5 to 8 for each observation; a vs c = * $P < 0.05$, a vs b = ** $P < 0.01$, b vs d = ** $P < 0.01$).

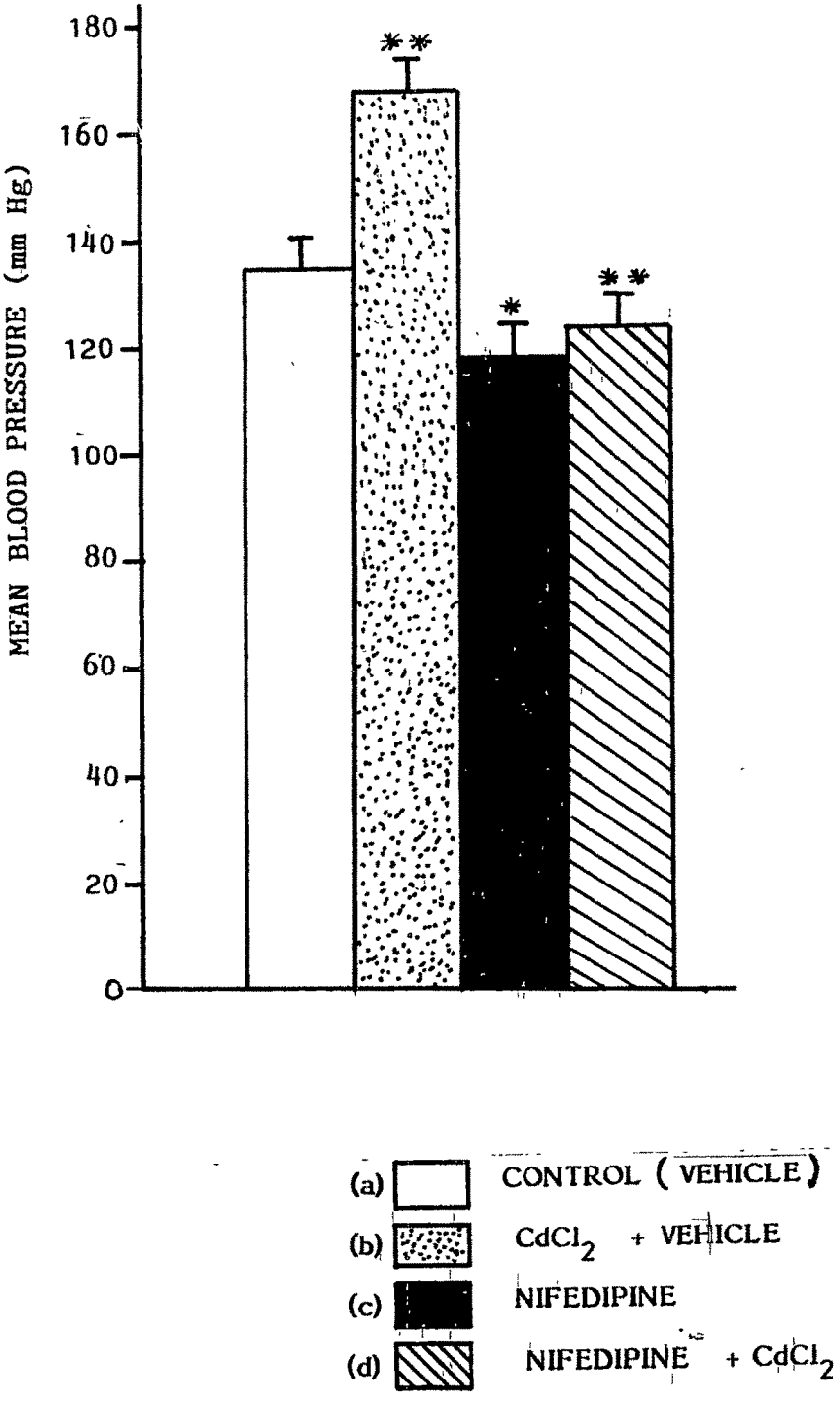
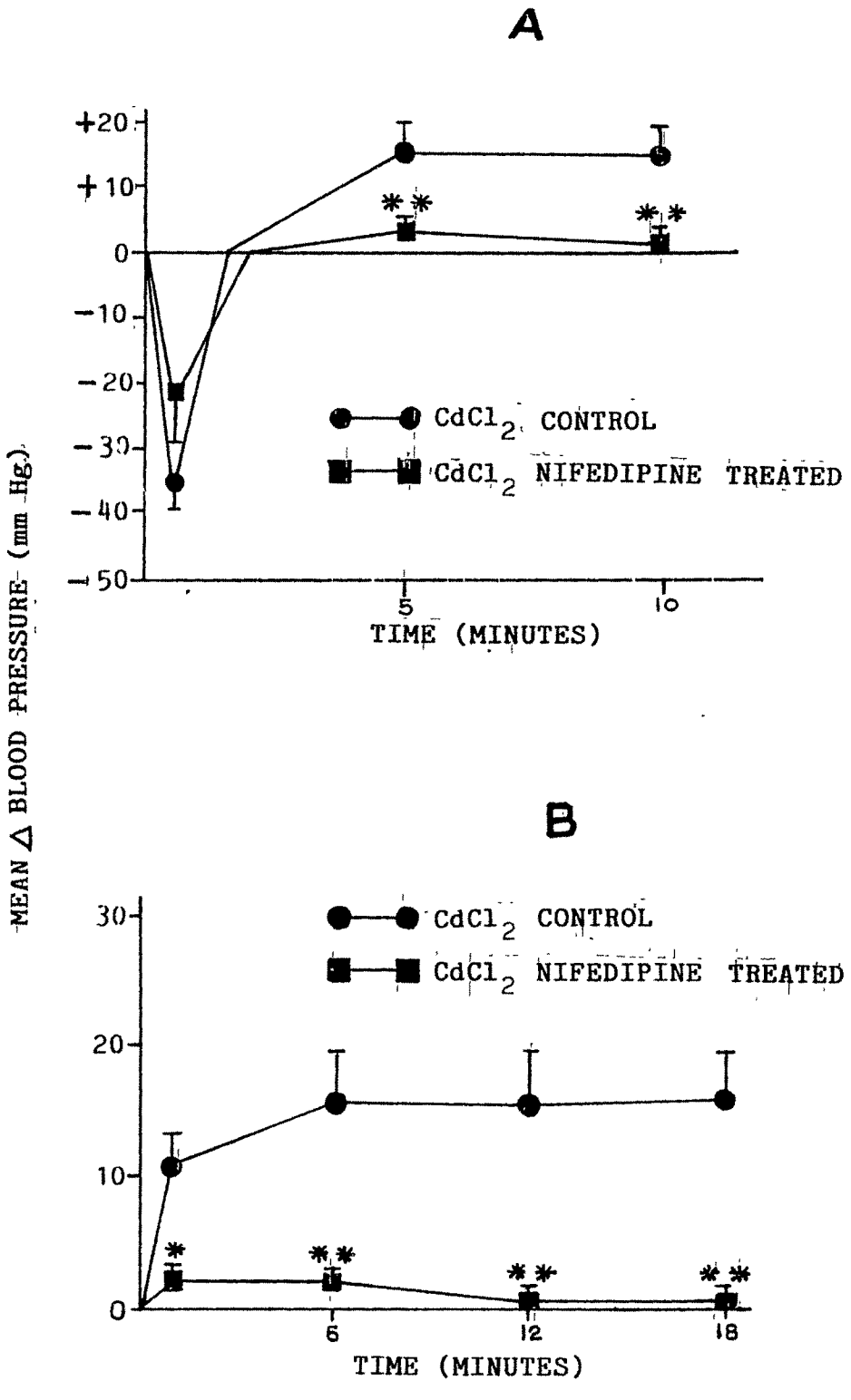

MEAN BLOOD PRESSURE (mm Hg)
180
160
140
120
100
80
60
40
20
0
**
*
**
(a) CONTROL (VEHICLE)
(b) CdCl2 + VEHICLE
(c) NIFEDIPINE
(d) NIFEDIPINE + CdCl2

Fig. 21 : Mean change in blood pressure produced by acute intravenous (A) and intraperitoneal (B) injections of $CdCl_2$ (1 mg/kg) in vehicle control and in nifedipine (10 mg/kg/day, p.o., for two weeks) treated anaesthetized rats. Abscissa indicates time in min and ordinate the change in blood pressure (mm Hg). Vertical lines represent SEM (n=4 for each observation; * $P < 0.05$ and ** $P < 0.01$; as compared with the corresponding control values).


A
+20
+10
0
−10
−20
−30
−40
−50
MEAN Δ BLOOD PRESSURE (mm Hg.)
$CdCl_2$ CONTROL
$CdCl_2$ NIFEDIPINE TREATED
5
10
TIME (MINUTES)
B
30
20
10
0
$CdCl_2$ CONTROL
$CdCl_2$ NIFEDIPINE TREATED
6
12
18
TIME (MINUTES)

2 mg) produced significant increase in the perfusion pressure (Fig. 22 and 23) which persisted for 30 to 45 minutes.

The basal perfusion pressure was significantly higher ($P < 0.05$) during perfusion with medium containing $CdCl_2$ (1 or 3 µg/ml (Fig. 24).

Phentolamine (10 µg/ml) could not prevent the increase in perfusion pressure due to intra-arterial $CdCl_2$ (1 mg) administration (Fig. 25).

Similarly acute reserpinization (5 mg/kg, i.p.) did not prevent the increase in perfusion pressure due to intra-arterial $CdCl_2$ (1 mg) administration (Fig. 25).

However, verapamil (50 and 100 µg/ml) could significantly ($P < 0.05$ and $P < 0.01$) prevent the increase in perfusion pressure due to intra-arterial $CdCl_2$ (1 mg) administration (Fig. 25).

4.2.1.2. ISOLATED RAT AORTA :

4.8×10^{-8} M and 4.8×10^{-7} M $CdCl_2$ produced a significant ($P < 0.05$ and $P < 0.01$) increase in the pD_2 value of KCl (Table X) with an increase in the maxima (Fig. 26 and 27). A higher concentration of $CdCl_2$ (1.44×10^{-5}M) produced a

significant rightward shift of dose-response curve with a depression of the maxima (Fig. 26).

4.8×10^{-8}M $CdCl_2$ produced a significant ($P<0.01$) increase in the pD_2 value of NA (Table X) with an increase in the maximal contractile response (Fig. 28). 4.8×10^{-7}M $CdCl_2$ did not produce significant change in the pD_2 value. An increased concentration of $CdCl_2$ (4.8×10^{-6}M) produced a parallel rightward shift of the dose-response curve of NA and decrease ($P<0.05$) in its pD_2 value. A still higher concentration of $CdCl_2$ (1.44×10^{-5}M) produced significant ($P<0.01$) rightward shift of dose-response curve with a depression of the maxima (Fig. 28).

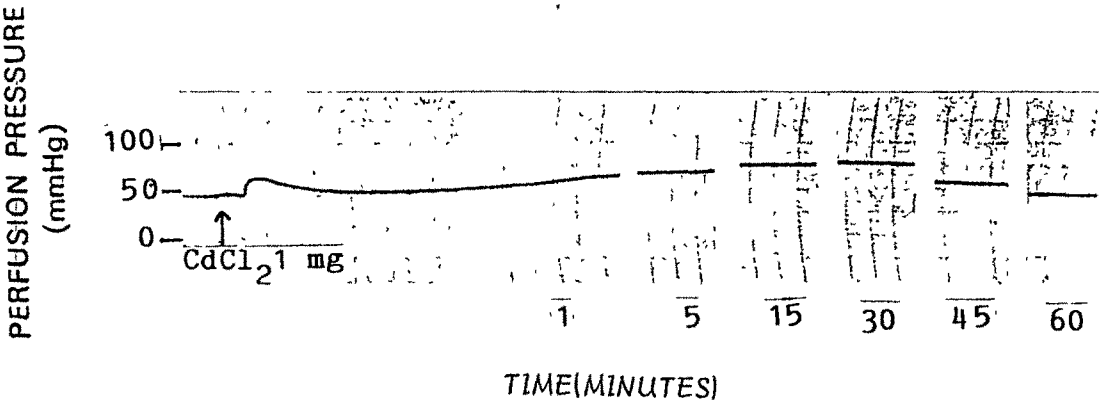
4.8×10^{-8}M, 4.8×10^{-7}M and 1.44×10^{-6}M $CdCl_2$ produced small contractile responses of the rat aorta. At a higher concentration, $CdCl_2$ (4.8×10^{-6}M) did not produce any contractile effect (Fig. 29A). The dose response curve of $CdCl_2$ was bell shaped (Fig. 30). The contractile effect of $CdCl_2$ on the aorta was completely absent after the removal of calcium from the bathing medium (Fig. 29B). Phentolamine (1.0×10^{-6}M) did not block the contractile effect of $CdCl_2$ (Fig. 29C).

4.2.1.3. <u>RAT PORTAL MESENTERIC VEIN</u> :

4.8×10^{-7}M $CdCl_2$ produced a significant ($P<0.01$) increase in the pD_2 value of KCl (Table XI) with an increase

Fig. 22 : Tracing of change in perfusion pressure of rat hindquarter preparation with intra-arterial administration of $CdCl_2$(1 mg). Abscissa indicates time in min following intra-arterial injection and ordinate the change in perfusion pressure (mm Hg).

Fig. 23 : Change in perfusion pressure of the rat hindquarter with intra-arterial administration of $CdCl_2$ (0.5, 1 and 2 mg). Vertical lines on histograms represent SEM (n=4 for each observation).


PERFUSION PRESSURE
(mmHg)
100
50
0
$CdCl_2$ 1 mg
1
5
15
30
45
60
TIME(MINUTES)

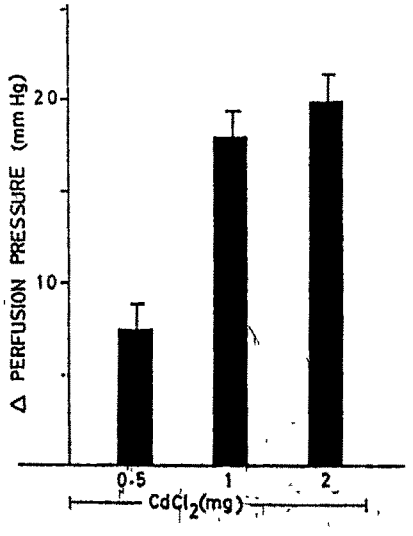

Δ PERFUSION PRESSURE (mm Hg)
20
10
0·5
1
2
$CdCl_2$(mg)

Fig. 24 : Mean basal perfusion pressure (mm Hg) in rat hindquarter preparations. White histogram indicates control, hatched and black histograms indicate basal perfusion pressure in the presence of 1 μg/ml and 3 μg/ml of $CdCl_2$ respectively. Vertical lines on histograms represent SEM (n=4 for each observation; * $P < 0.05$ as compared with control).

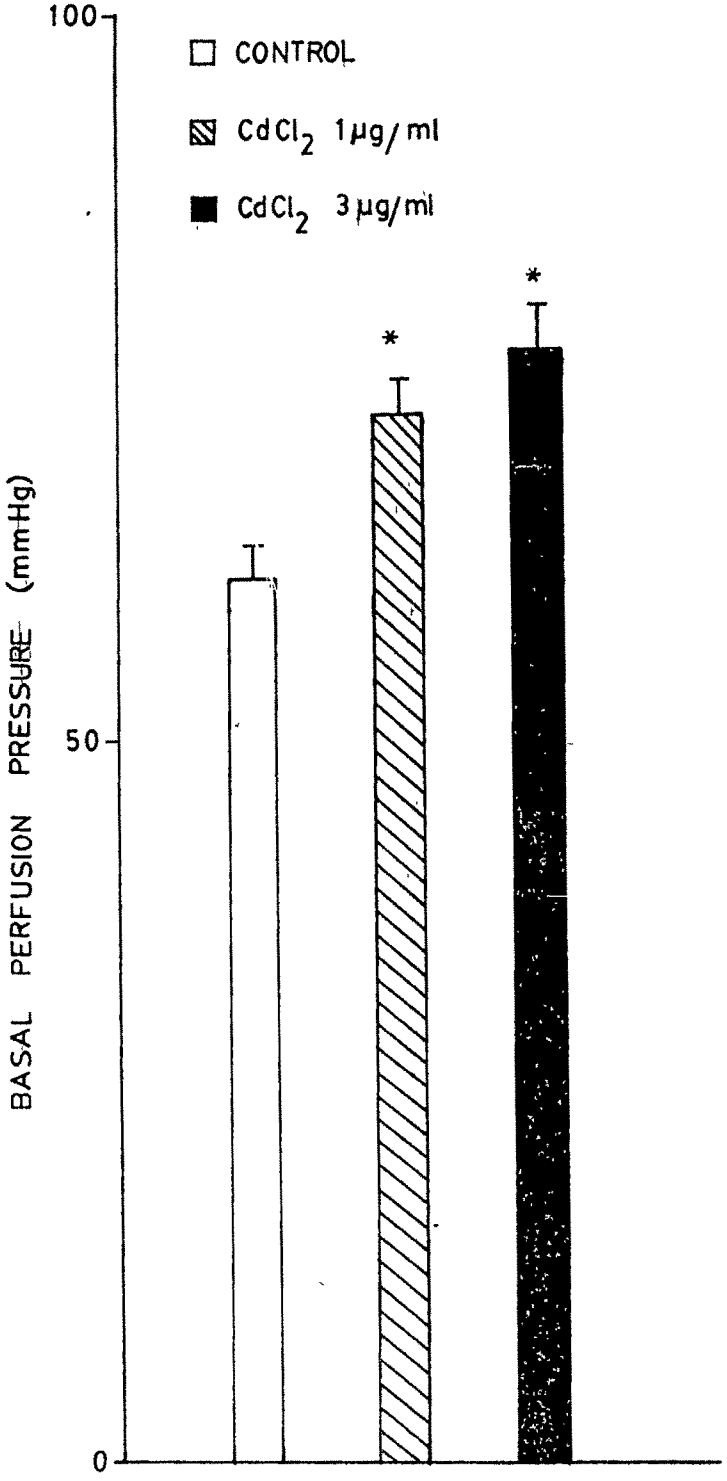

100
50
0
CONTROL
CdCl2 1µg/ml
CdCl2 3µg/ml
BASAL PERFUSION PRESSURE (mmHg)
*
*

Fig. 25 : Mean change in perfusion pressure (mm Hg) of rat hindquarter preparations due to intra-arterial administration of $CdCl_2$(1 mg). White histogram indicates control perfusion pressure due to $CdCl_2$. Black, circled and vertically hatched histograms indicate the change in perfusion pressure due to intra-arterial $CdCl_2$ administration in the presence of phentolamine (10 µg/ml) verapamil (50 µg/ml) and verapamil (100 µg/ml) respectively. Diagonally hatched histogram indicates the change in perfusion pressure due to intra-arterial $CdCl_2$ in reserpinized (reserpine 5 mg/kg, i.p., 24 hrs before the experiments) preparation. Vertical lines on histograms represent SEM (n=4 for each observation; * $P < 0.05$ and ** $P < 0.01$ as compared with control).

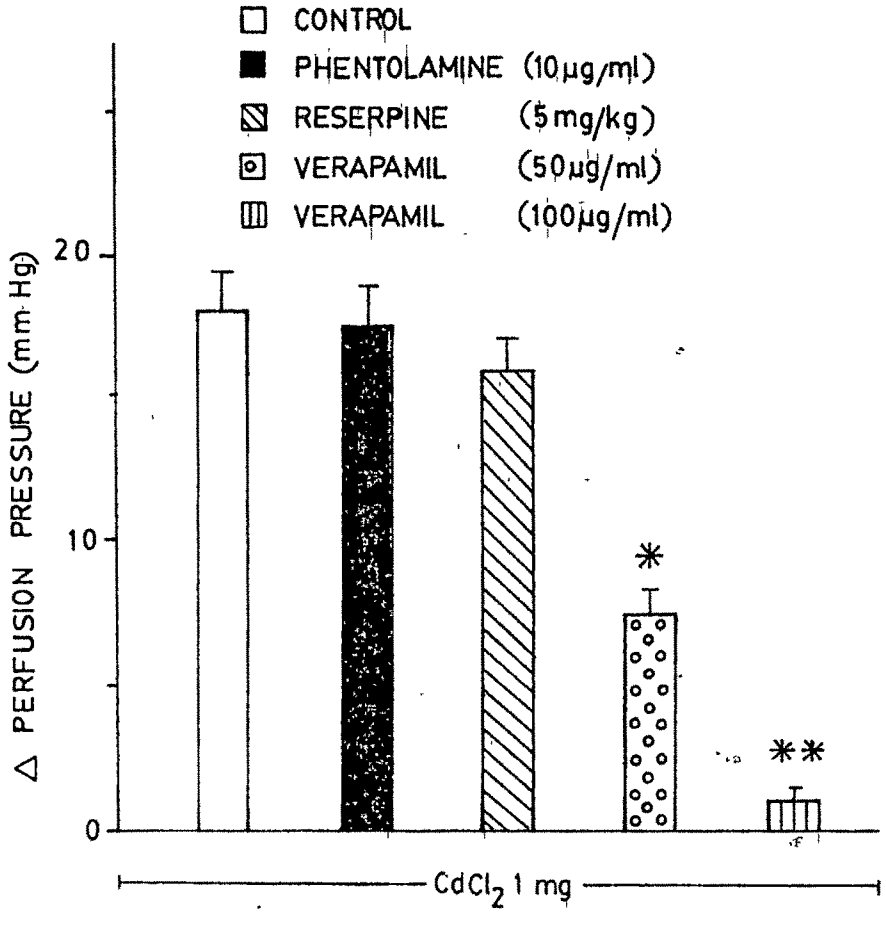

CONTROL
PHENTOLAMINE (10µg/ml)
RESERPINE (5mg/kg)
VERAPAMIL (50µg/ml)
VERAPAMIL (100µg/ml)
Δ PERFUSION PRESSURE (mm Hg)
20
10
0
*
**
CdCl2 1 mg

Table X : pD_2 values of KCl and NA in the absence and presence of different concentrations of $CdCl_2$ in rat isolated aorta (n=5)

Agonist	Mean pD_2 value (±SEM)				
	Control	$CdCl_2$			
		4.8×10^{-8}M	4.8×10^{-7}M	4.8×10^{-6}M	1.44×10^{-5}M
KCl	1.48±0.05	1.74±0.05*	1.93±0.04**	1.52±0.04	maxima suppressed
NA	6.90±0.02	7.19±0.06**	6.93±0.03	6.76±0.03*	"

* $P < 0.05$

** $P < 0.01$

Fig. 26 : Cumulative dose-response curves of KCl in rat isolated aorta. Abscissa indicates the log molar concentration of KCl and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-8}M (△—△), 4.8×10^{-7}M (□—□), 4.8×10^{-6}M (○—○) and 1.44×10^{-5}M (■—■) $CdCl_2$ are shown. Vertical lines represent SEM (n=5 for each observation; * $P < 0.05$ and ** $P < 0.01$ as compared with the corresponding control response).

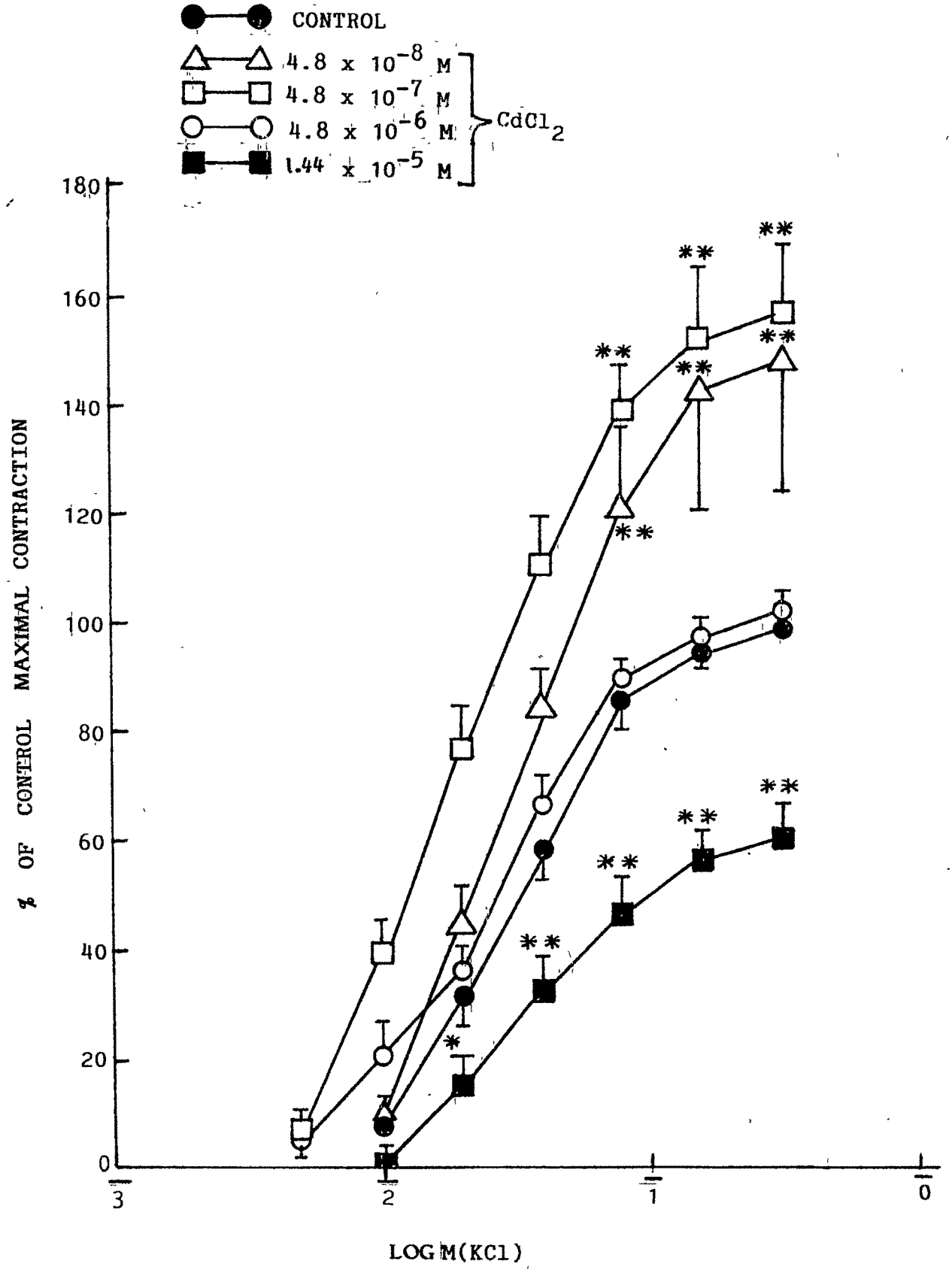
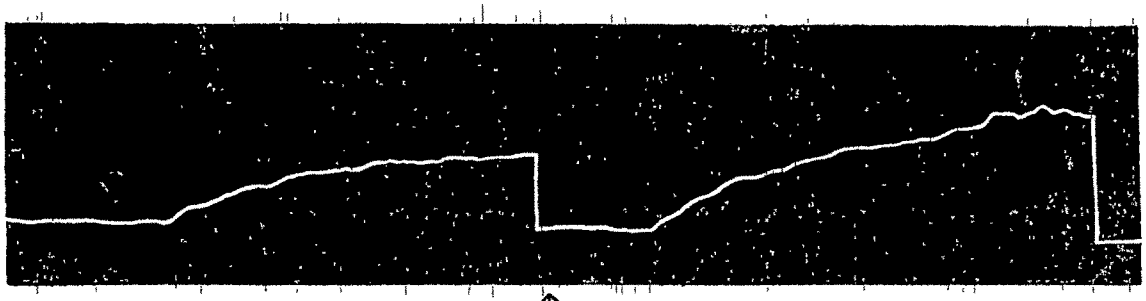

CONTROL
4.8 x 10⁻⁸ M
4.8 x 10⁻⁷ M
4.8 x 10⁻⁶ M
1.44 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
180
160
140
120
100
80
60
40
20
0
3
2
1
0
LOG M(KCl)

Fig. 27 : Kymographic tracing of cumulative concentration-response effect of KCl in isolated rat aorta in the absence and in the presence of $CdCl_2$ (4.8×10^{-7}M) in the bathing medium. Note that low concentration of $CdCl_2$ produced a marked potentiation of KCl response.



1×10^{-2}M -3.16×10^{-1}M

$CdCl_2$

4.8×10^{-7}M

5.6×10^{-3}M - 3.16×10^{-1}M

Fig. 28 : Cumulative dose-response curves of NA in the isolated rat aorta. Abscissa indicates the log molar concentration of NA and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-8}M(△—△), 4.8×10^{-7}M (□—□), 4.8×10^{-6}M (○—○) and 1.44×10^{-5}M (■—■) $CdCl_2$ are shown. Vertical lines represent SEM (n=5 for each observation; * $P < 0.05$ and ** $P < 0.01$, as compared with the corresponding control responses).

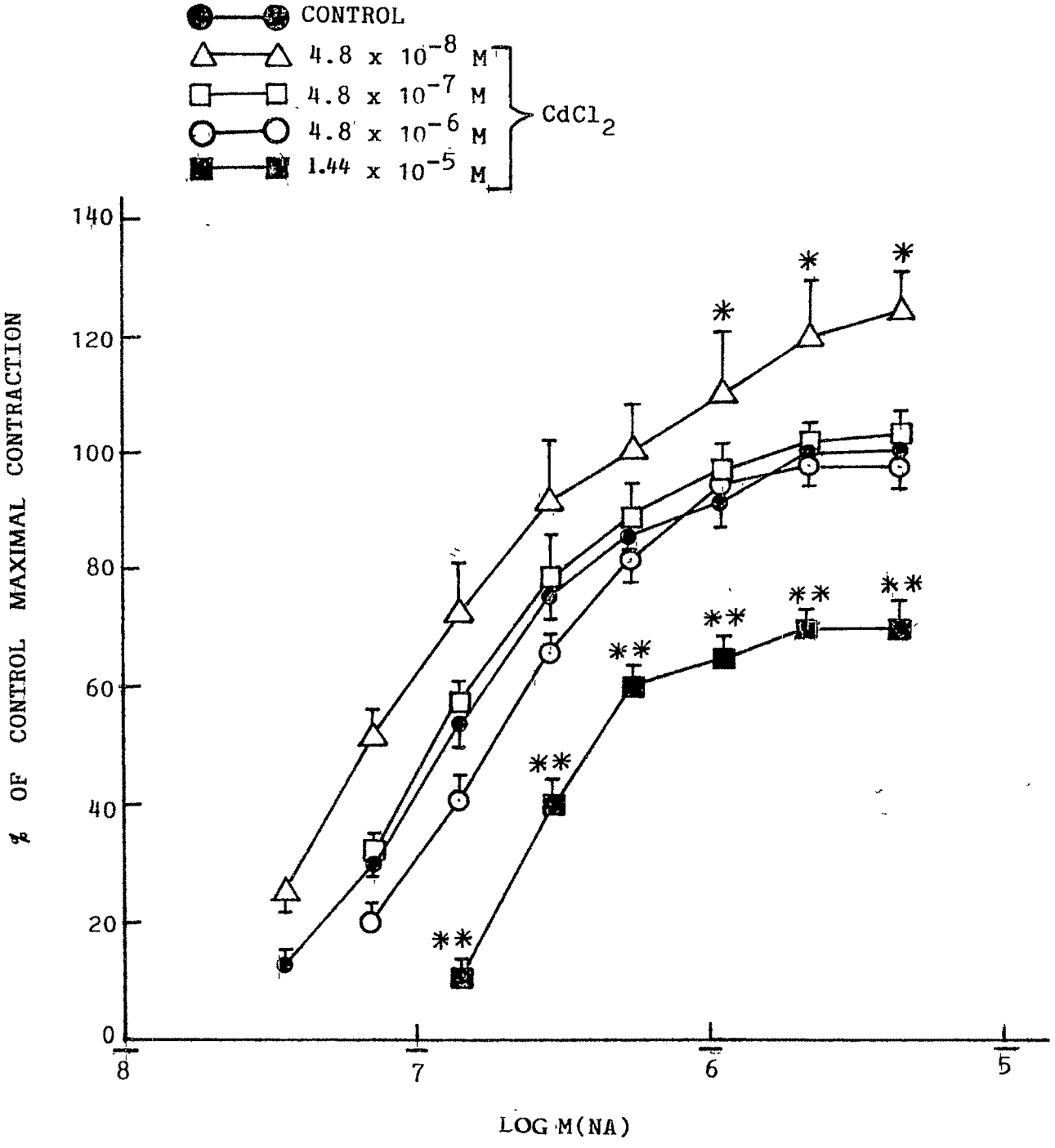
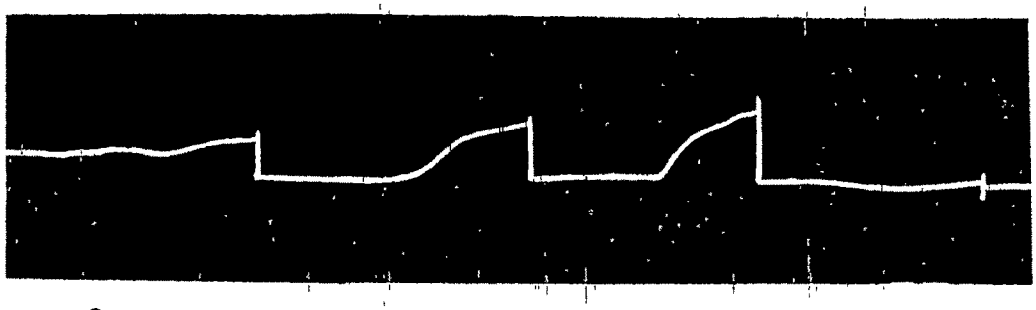

CONTROL
4.8 x 10⁻⁸ M
4.8 x 10⁻⁷ M
4.8 x 10⁻⁶ M
1.44 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
140
120
100
80
60
40
20
0
8
7
6
5
LOG M(NA)

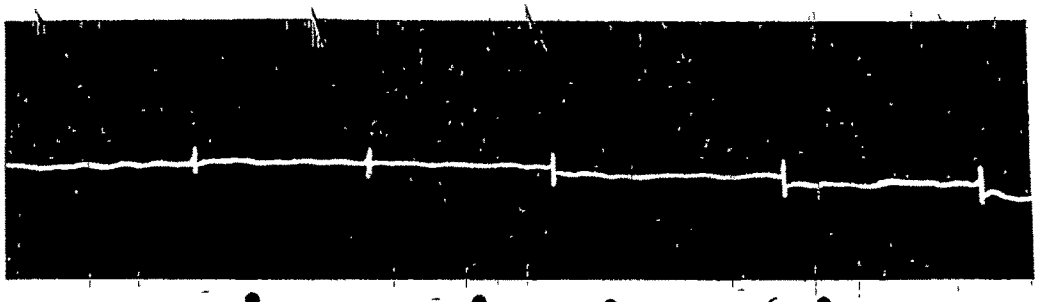
Fig. 29 : Kymographic tracing of the effect of different concentrations of $CdCl_2$ on the rat isolated aorta. (Control, A, in the Ca^{++} free medium, B, and in the presence of phentolamine 1×10^{-6}M, C.) Note that lower concentrations (4.8×10^{-8}M, 4.8×10^{-7}M and 1.44×10^{-6}M) produced contractile effect while a higher concentration did not produce any effect. Also note that there is absence of contractile effect of $CdCl_2$ in calcium-free medium. Phentolamine did not block the contractile effect of $CdCl_2$.

A



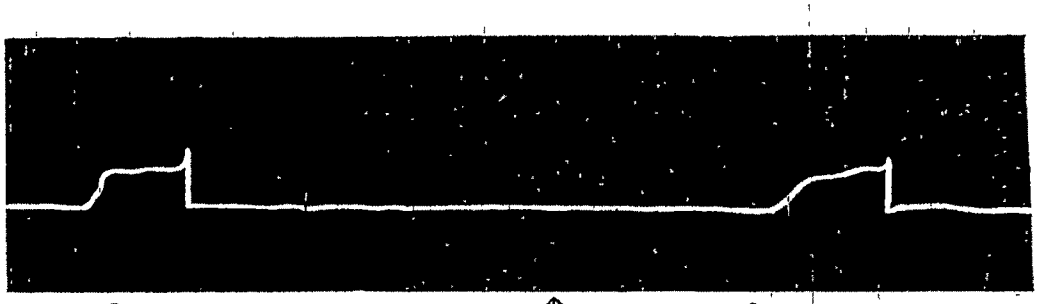
4.8×10^{-8} M 4.8×10^{-7} M 1.44×10^{-6}M 4.8×10^{-6}M

B



4.8×10^{-8}M 4.8×10^{-7}M 1.44×10^{-6}M 4.8×10^{-6}M

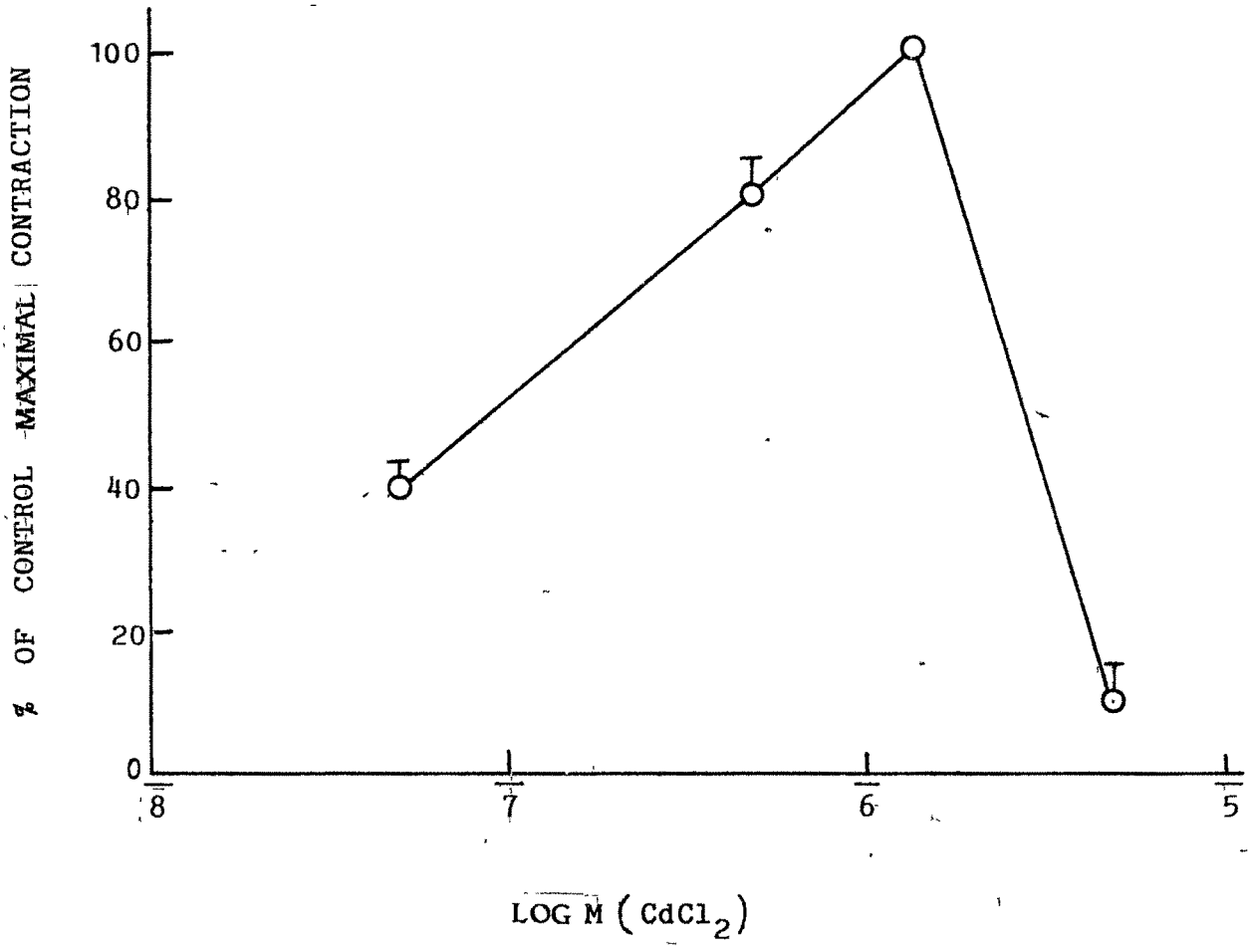
C



1.44×10^{-6}M PHENTOLAMINE 1.44×10^{-6}M

1×10^{-6} M

Fig. 30 : Dose-response curve of $CdCl_2$ (4.8×10^{-8}M to 4.8×10^{-6}M) in rat isolated aorta. Abscissa indicates the log molar concentration of $CdCl_2$ and ordinate the % of maximal contraction. Vertical lines represent SEM (n=4 for each observation).


% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
8
7
6
5
LOG M ($CdCl_2$)

in the maxima (Fig. 31). Increased concentrations of $CdCl_2$ (4.8×10^{-6}M and 1.44×10^{-5}M) did not produce any change in the pD_2 value. However, a still higher concentration of $CdCl_2$ (4.8×10^{-5}M) produced a rightward shift of the dose-response curve with a significant ($P < 0.001$) suppression of the maxima (Fig. 31).

4.8×10^{-7}M and 4.8×10^{-6}M $CdCl_2$ did not produce significant change in the pD_2 value of NA (Table XI). However, a higher concentration of $CdCl_2$ (4.8×10^{-5}M) produced a significant ($P < 0.001$) rightward shift of the dose-response curve of NA with a suppression of the maxima (Fig. 32).

4.2.1.4. RAT VAS DEFERENS :

1.44×10^{-8}M $CdCl_2$ produced significant increase in the pD_2 value ($P < 0.05$) of KCl (Table XII) with an increase in the maxima (Fig. 33). However, an increased concentration of $CdCl_2$ (4.8×10^{-7}M) did not produce any change in the pD_2 value of KCl. With a still higher concentration of $CdCl_2$ (4.8×10^{-6}M), there was a significant ($P < 0.001$) rightward shift in the dose-response curve of KCl with a suppression of the maxima (Fig. 33).

1.44×10^{-8}M $CdCl_2$ produced an enhancement of the tonic phase of the contraction produced by KCl (Fig. 34).

4.8×10^{-9}M and 1.44×10^{-8}M $CdCl_2$ did not produce any change in the pD_2 value of NA (Table XII). However, with a higher concentration of $CdCl_2$ (4.8×10^{-6}M), there was a significant ($P < 0.05$ and $P < 0.01$) rightward shift of the dose-response curve with a depression of the maxima (Fig. 35).

4.2.1.5. RAT ANOCOCCYGEUS MUSCLE :

4.8×10^{-6}M $CdCl_2$ caused reduction ($P < 0.05$) in the pD_2 value (Table XIII) of KCl without any change in the maxima. However, with higher concentrations of $CdCl_2$ (1.44×10^{-5}M and 4.8×10^{-5}M) there was a rightward shift of the dose-response curve of KCl with a significant ($P < 0.01$ and $P < 0.001$) suppression of the maxima (Fig. 36). When the calcium concentration in the perfusion fluid was reduced to 25%, 4.8×10^{-6}M and 1.44×10^{-5}M $CdCl_2$ produced a highly significant ($P < 0.001$) rightward shift of the dose-response curve and suppression of the maxima (Fig. 37).

4.8×10^{-6}M $CdCl_2$ did not produce a significant change in the pD_2 value of NA (Table XIII). With an increase in the concentration of $CdCl_2$ (1.44×10^{-5}M) there was a significant ($P < 0.05$) decrease in the pD_2 value of NA (Table XIII) without any change in the maxima. A still higher concentration of

$CdCl_2$ (4.8×10^{-5}M) produced a significant ($P < 0.01$ and $P < 0.001$) rightward shift of the dose-response curve and suppression of the maxima (Fig. 38). When the calcium concentration in the perfusion fluid was reduced to 25%, 4.8×10^{-6}M and 1.44×10^{-5}M $CdCl_2$ produced a significant ($P < 0.05$ and $P < 0.01$) decrease in the pD_2 (Table XIII) value of NA. With a still higher concentration of $CdCl_2$ (4.8×10^{-5}M) there was a highly significant ($P < 0.001$) suppression of the maxima (Fig. 39).

4.2.2. CHRONIC EXPERIMENTS :

4.2.2.1. HINDQUARTER PERFUSION :

Chronic treatment of rats with $CdCl_2$ (0.1 and 0.5 mg/kg/day, i.p.) for two weeks did not produce any significant change in the basal perfusion pressure. However, in preparations obtained from rats treated with a higher dose (1 mg/kg, i.p.) for two weeks, there was a significant ($P < 0.05$) increase in the basal perfusion pressure (Fig. 40).

With chronic $CdCl_2$ (0.1 and 0.5 mg/kg/day,i.p.,for two weeks) treatment, there was no significant change in the perfusion pressure of the hindquarter of rats, to various doses (10, 20 and 40 ug) of NA. However, with a higher dose of $CdCl_2$ (1 mg/kg/day, i.p.,for two weeks), there was a significant ($P < 0.05$) increase in the perfusion pressure to NA (Fig. 41).

Table XI : pD_2 values of KCl and NA in the absence and presence of different concentrations of $CdCl_2$ in rat isolated portal mesenteric vein (n=5).

Agonist	Mean pD_2 value (±SEM)					P value
	Control	$CdCl_2$				
		4.8×10^{-7} M	4.8×10^{-6} M	1.44×10^{-5} M	4.8×10^{-5} M	
KCl	1.56±0.03	1.93±0.05**	1.61±0.05	1.51±0.03	Maxima suppressed	<0.01
NA	6.20±0.11	6.47±0.10	6.41±0.08	-	"	> 0.05

Fig. 31 : Dose-response curves of KCl on rat isolated portal mesenteric vein. Abscissa indicates the log molar concentration of KCl and ordinate the % of control maximal contraction. Control response (●—●) and those in presence of 4.8×10^{-7}M (□—□), 4.8×10^{-6}M (○—○), 1.44×10^{-5}M (■—■), 4.8×10^{-5}M (▲—▲) $CdCl_2$ are shown. Vertical lines represent SEM (n=5 for each observation; * $P < 0.05$ and ** $P < 0.001$ as compared with the corresponding control responses).

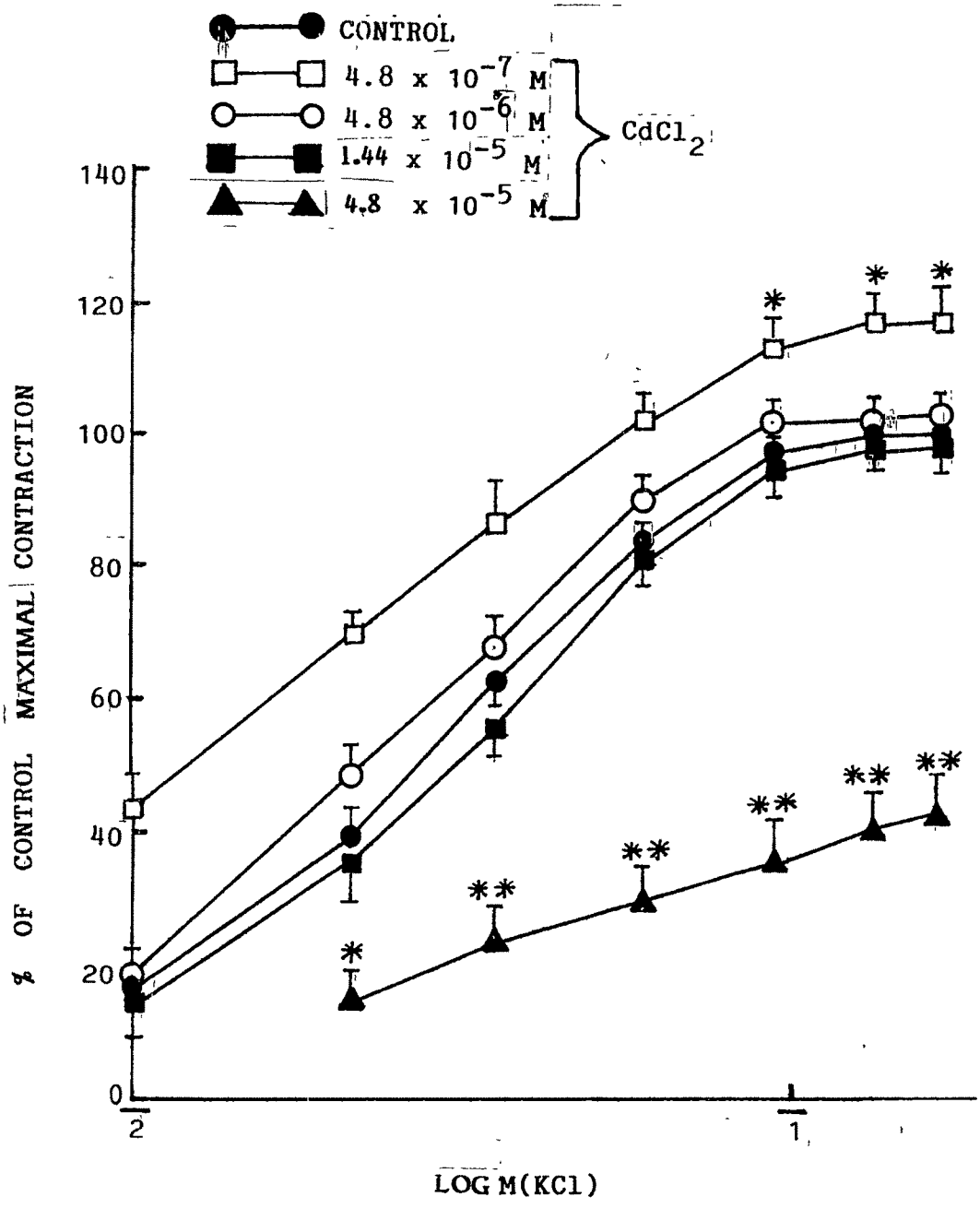

CONTROL
4.8 x 10⁻⁷ M
4.8 x 10⁻⁶ M
1.44 x 10⁻⁵ M
4.8 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
140
120
100
80
60
40
20
0
2
1
LOG M(KCl)

Fig. 32 : Cumulative dose-response curves of NA on rat isolated portal mesenteric vein. Abscissa indicates the log molar concentration of NA and ordinate the % of control maximal contraction. Control responses (●—●) and those in the presence of 4.8×10^{-7}M (□—□), 4.8×10^{-6}M (○—○), 4.8×10^{-5}M (▲—▲) $CdCl_2$ are shown. Vertical lines represent SEM (n=5 for each observation; * $P < 0.05$ and ** $P < 0.001$ as compared with control).

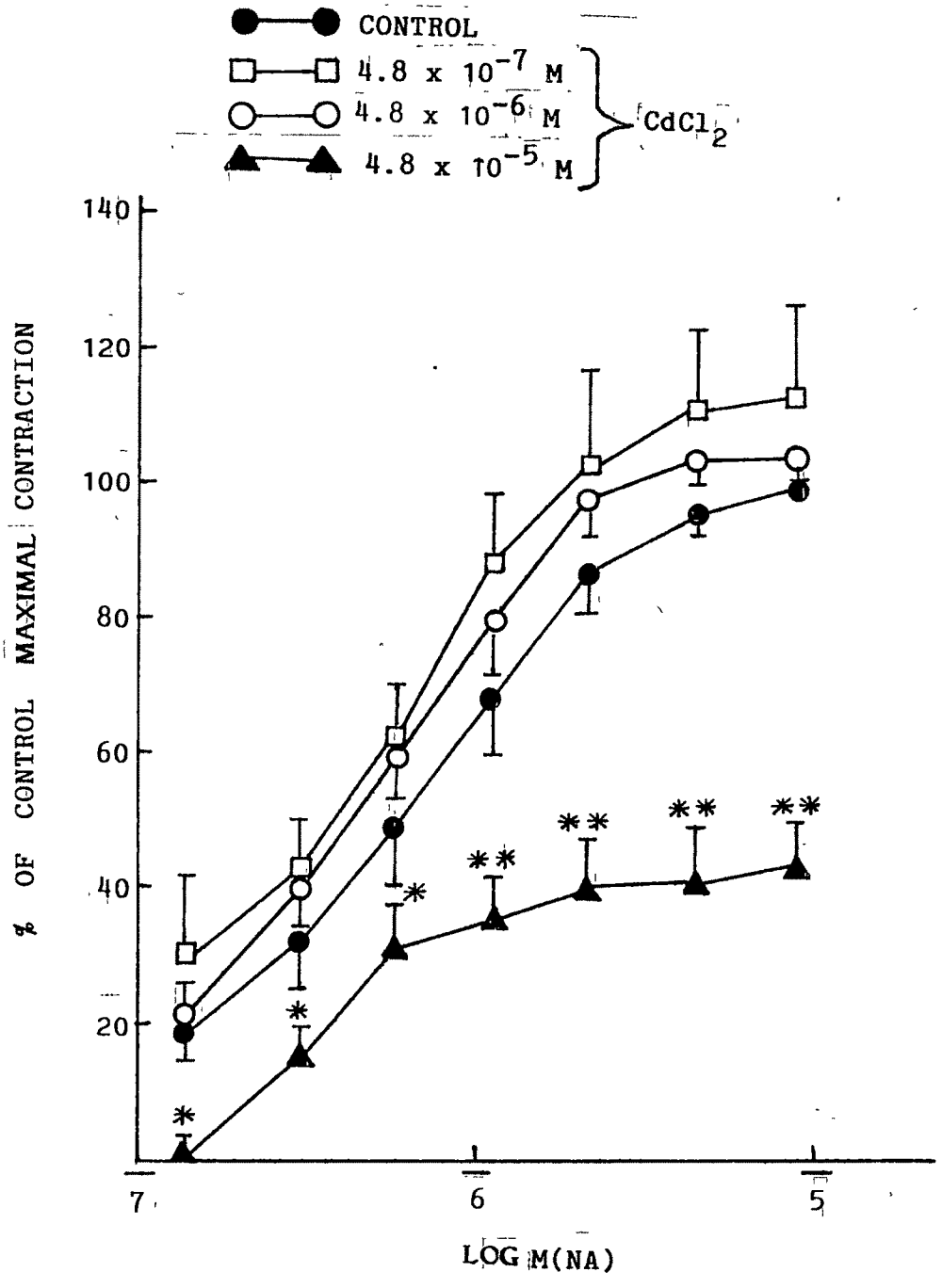

CONTROL
4.8 x 10⁻⁷ M
4.8 x 10⁻⁶ M
4.8 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
140
120
100
80
60
40
20
7
6
5
LOG M(NA)

Table XII : pD_2 values of KCl and NA in the absence and presence of different concentrations of $CdCl_2$ in rat isolated vas deferens (n=5)

Agonist	Mean pD_2 value (±SEM)					P value
	Control	$CdCl_2$				
		4.8×10^{-9}M	1.44×10^{-8}M	4.8×10^{-7}M	4.8×10^{-6}M	
KCl	1.51±0.04	1.55±0.06	1.68±0.03*	1.41±0.11	Maxima suppressed	<0.05
NA	5.69±0.04	5.72±0.05	5.61±0.05	-	"	> 0.05

Fig. 33 : Dose-response curves of KCl on rat isolated vas deferens. Abscissa indicates the log molar concentration of KCl and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-9}M (▲—▲), 1.44×10^{-8}M (◪—◪), 4.8×10^{-7}M (□—□) and 4.8×10^{-6}M (○—○) $CdCl_2$ are shown. Vertical lines represent SEM (n=5 for each observation; * $P < 0.05$, and ** $P < 0.001$ as compared with the corresponding control responses).

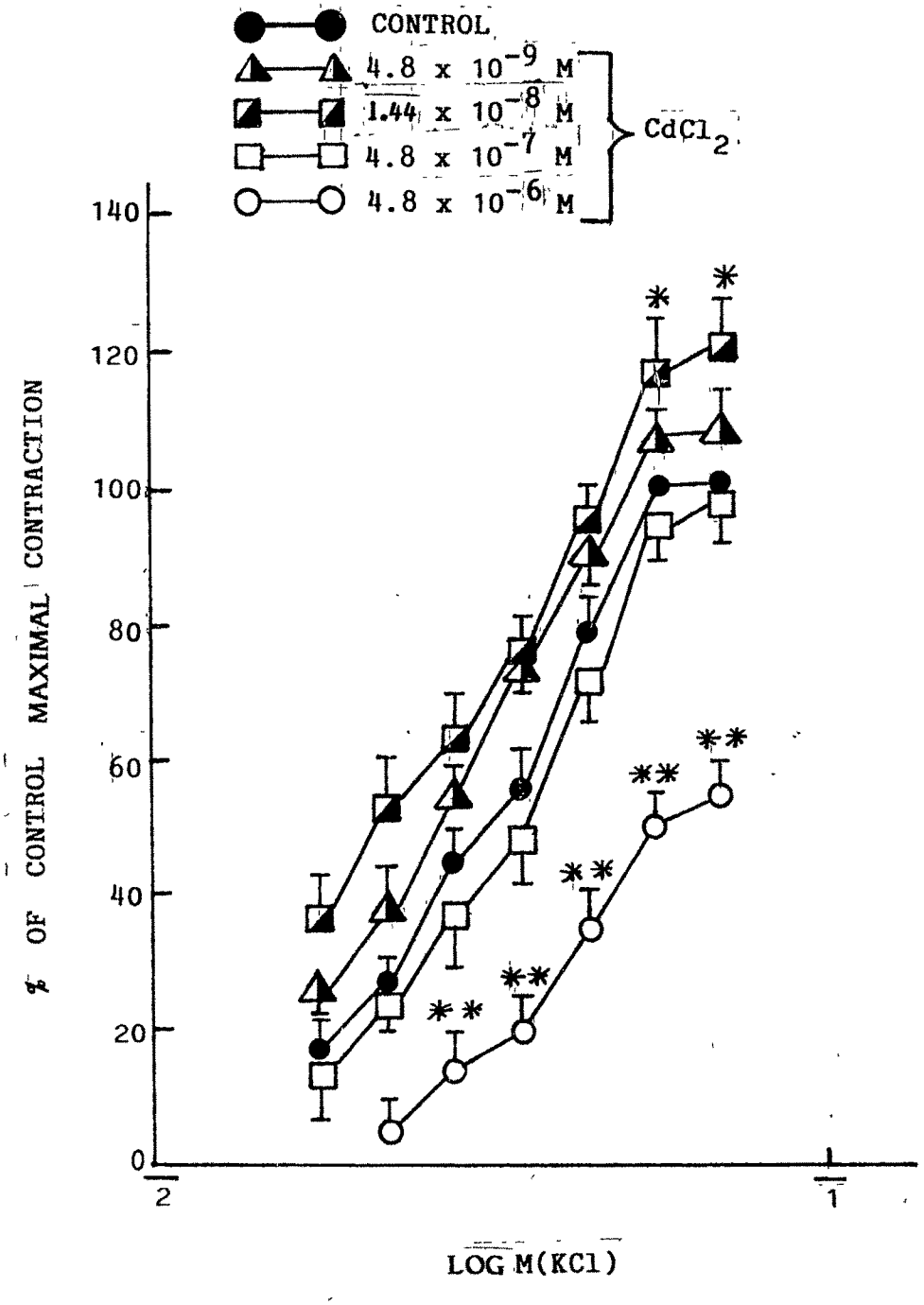

CONTROL
4.8 x 10⁻⁹ M
1.44 x 10⁻⁸ M
4.8 x 10⁻⁷ M
4.8 x 10⁻⁶ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
140
120
100
80
60
40
20
0
2
1
LOG M(KCl)

Fig. 34 : Kymographic tracing of the contractile responses to KCl (3.5×10^{-2}M and 4.6×10^{-2}M) on rat isolated vas deferens in the absence and presence of a low concentration of $CdCl_2$ (1.44×10^{-8}M). Note that the tonic phase of the contractile response to KCl was enhanced in the presence of $CdCl_2$ (1.44×10^{-8}M).

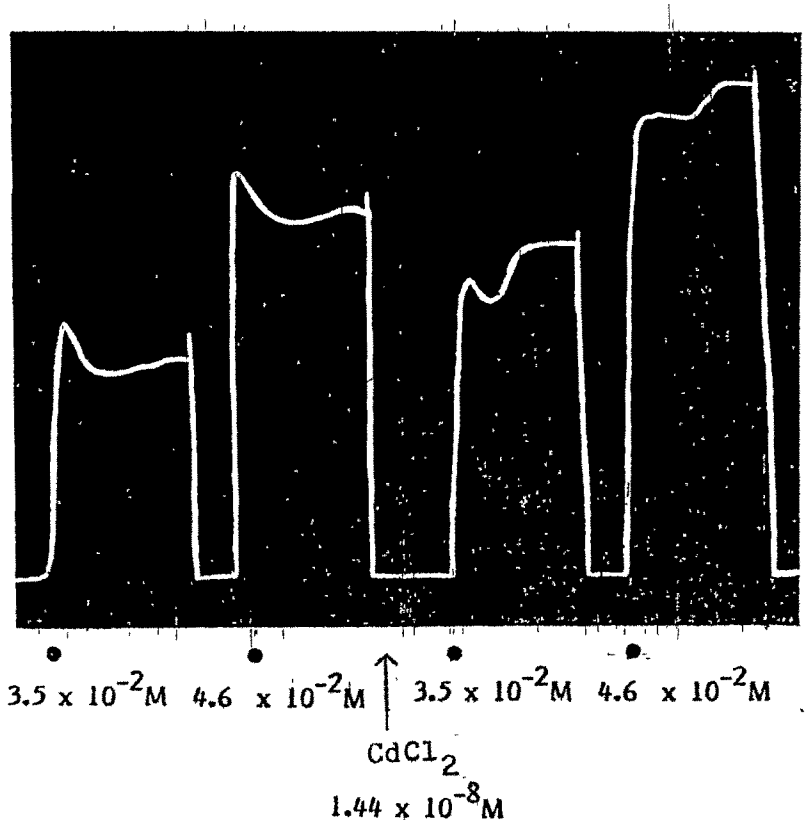

3.5 x 10^{-2}M
4.6 x 10^{-2}M
3.5 x 10^{-2}M
4.6 x 10^{-2}M
CdCl$_2$
1.44 x 10^{-8}M

Fig. 35 : Dose-response curves of NA in rat isolated vas deferens. Abscissa indicates the log molar concentration and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-9}M, (▲—▲), 1.44×10^{-8}M (◪—◪), 4.8×10^{-6}M (O—O) $CdCl_2$ are shown. Vertical lines represent SEM (n=5 for each observation; * $P < 0.05$ and ** $P < 0.01$ as compared with the corresponding control responses).

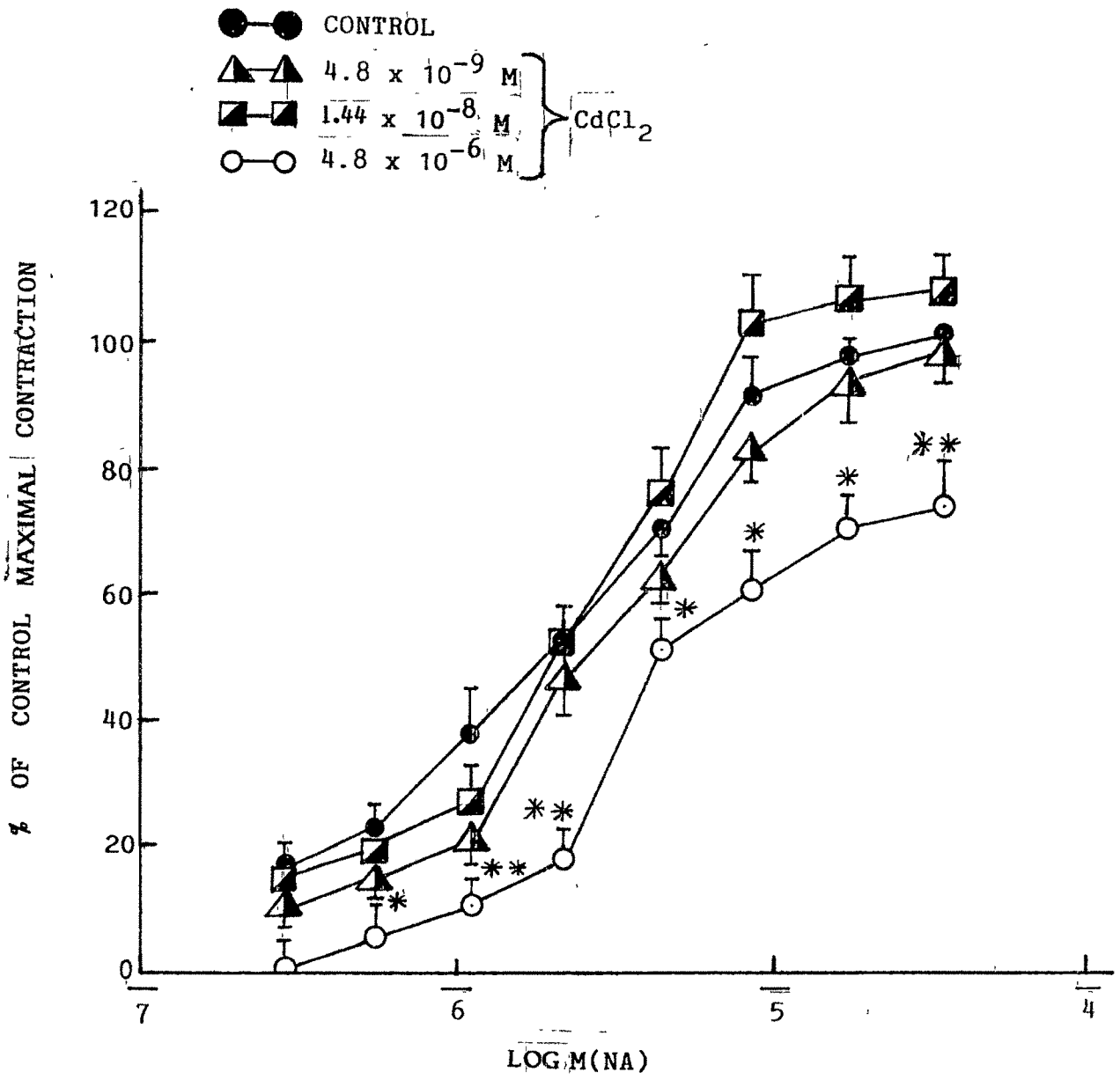

CONTROL
4.8 x 10⁻⁹ M
1.44 x 10⁻⁸ M
4.8 x 10⁻⁶ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
120
100
80
60
40
20
0
7
6
5
4
LOG M(NA)

Table XIII : pD_2 values of NA and KCl in the absence and presence of different concentrations of $CdCl_2$ in rat isolated anococcygeus muscle (n=5)

Agonist	Mean pD_2 value (±SEM)				P value
	Control	$CdCl_2$			
		4.8×10^{-6}M	1.44×10^{-5}M	4.8×10^{-5}M	
KCl					
A	1.49±0.04	1.35±0.03*	Maxima suppressed	Maxima suppressed	<0.05
B	1.30±0.03	Maxima suppressed	"	"	
NA					
A	6.19±0.09	5.96±0.11	5.79±0.11*	"	<0.05
B	5.91±0.08	5.61±0.08*	5.26±0.04**	" <0.05	<0.01

A = Normal Ca^{++} concentration in Krebs (2.52 mM)

B = Ca^{++} concentration reduced to 25% (0.63 mM)

Fig. 36 : Dose-response curves of KCl in rat isolated anococcygeus muscle. Abscissa indicates the log molar concentration of KCl and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-6}M (○—○), 1.44×10^{-5}M (■—■), 4.8×10^{-5}M (▲—▲) $CdCl_2$ are shown. Vertical lines represent SEM (n=5 for each observation; ** $P < 0.01$, and *** $P < 0.001$ as compared with the corresponding control responses).

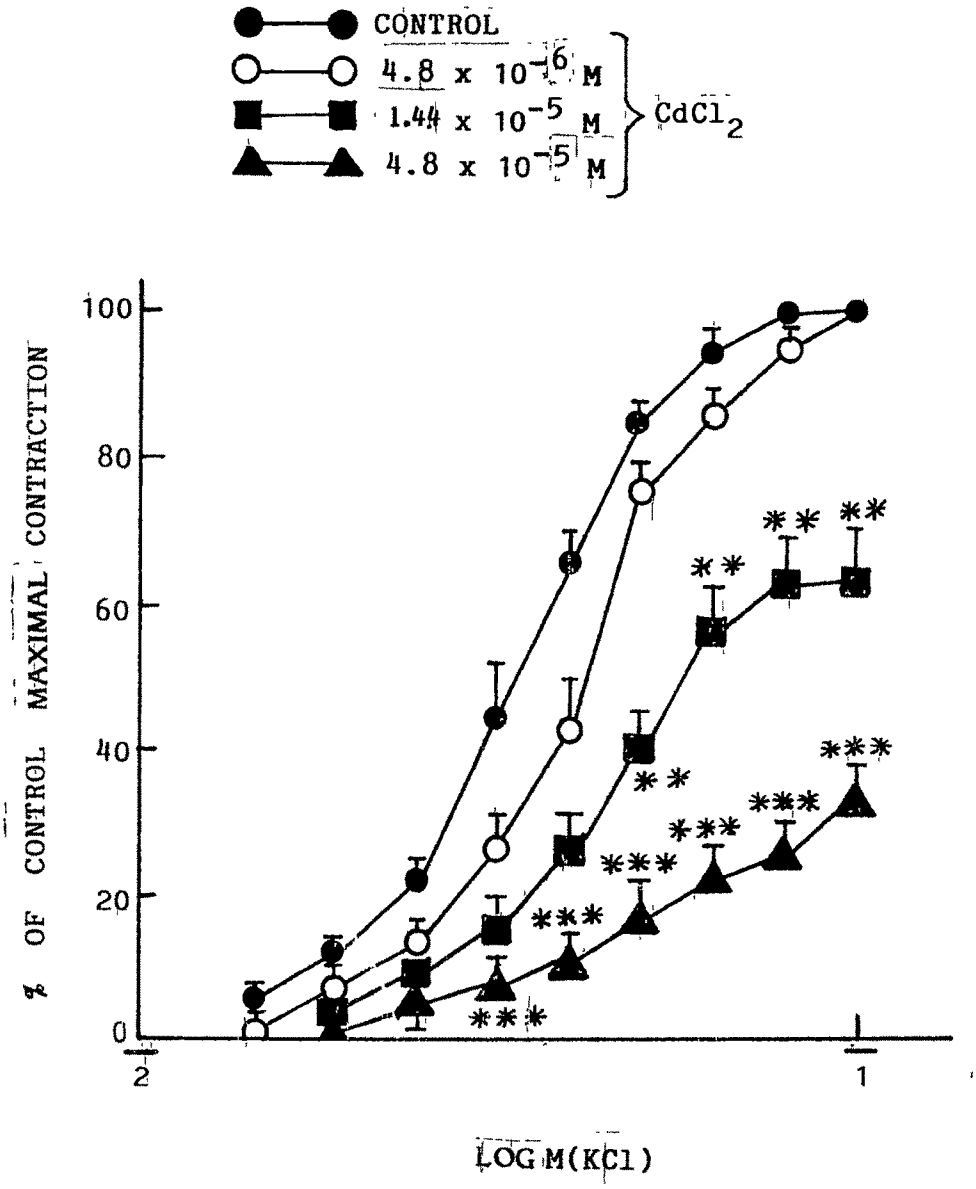

CONTROL
4.8 x 10⁻⁶ M
1.44 x 10⁻⁵ M
4.8 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
2
1
LOG M(KCl)

Fig. 37 : Dose-response curves of KCl in rat isolated anococcygeus muscle. Abscissa indicates the log molar concentration of KCl and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-6}M (○—○), 1.44×10^{-5}M (■—■) $CdCl_2$ elicited in reduced (0.63 mM) calcium concentration in the medium. Vertical lines represent SEM (n=5 for each observation; ** P < 0.01 and *** P < 0.001 as compared with the corresponding control responses).

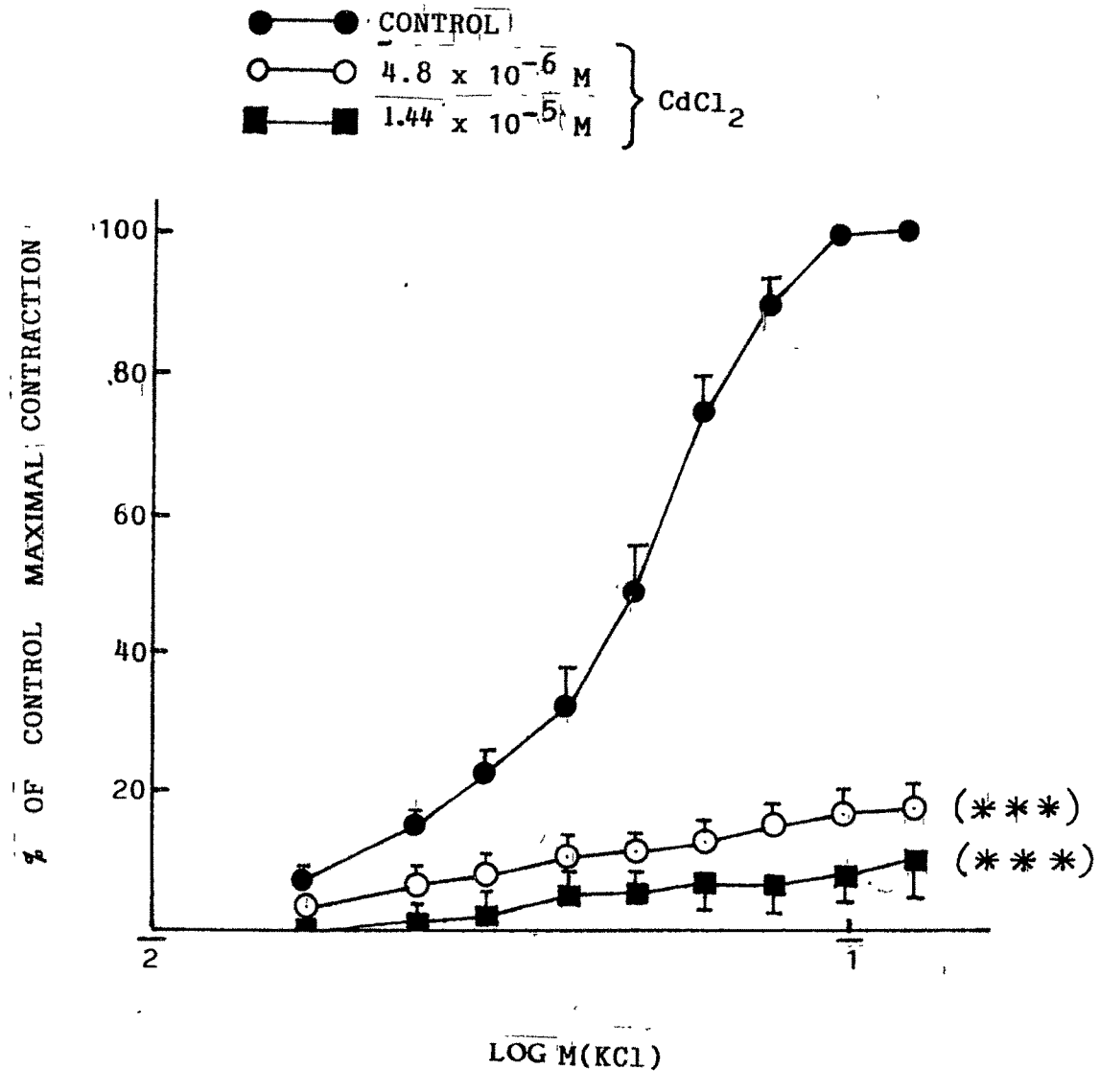

CONTROL
4.8 x 10⁻⁶ M
1.44 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
2
1
(***)
(***)
LOG M(KCl)

Fig. 38 : Dose-response curves of NA in rat isolated anococcygeus muscle. Abscissa indicates log molar concentration of NA and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-6}M (○—○), 1.44×10^{-5}M (■—■) and 4.8×10^{-5}M (▲—▲) are shown. Vertical lines represent SEM (n=5 for each observation; ** $P < 0.01$ and *** $P < 0.001$ as compared with control responses).

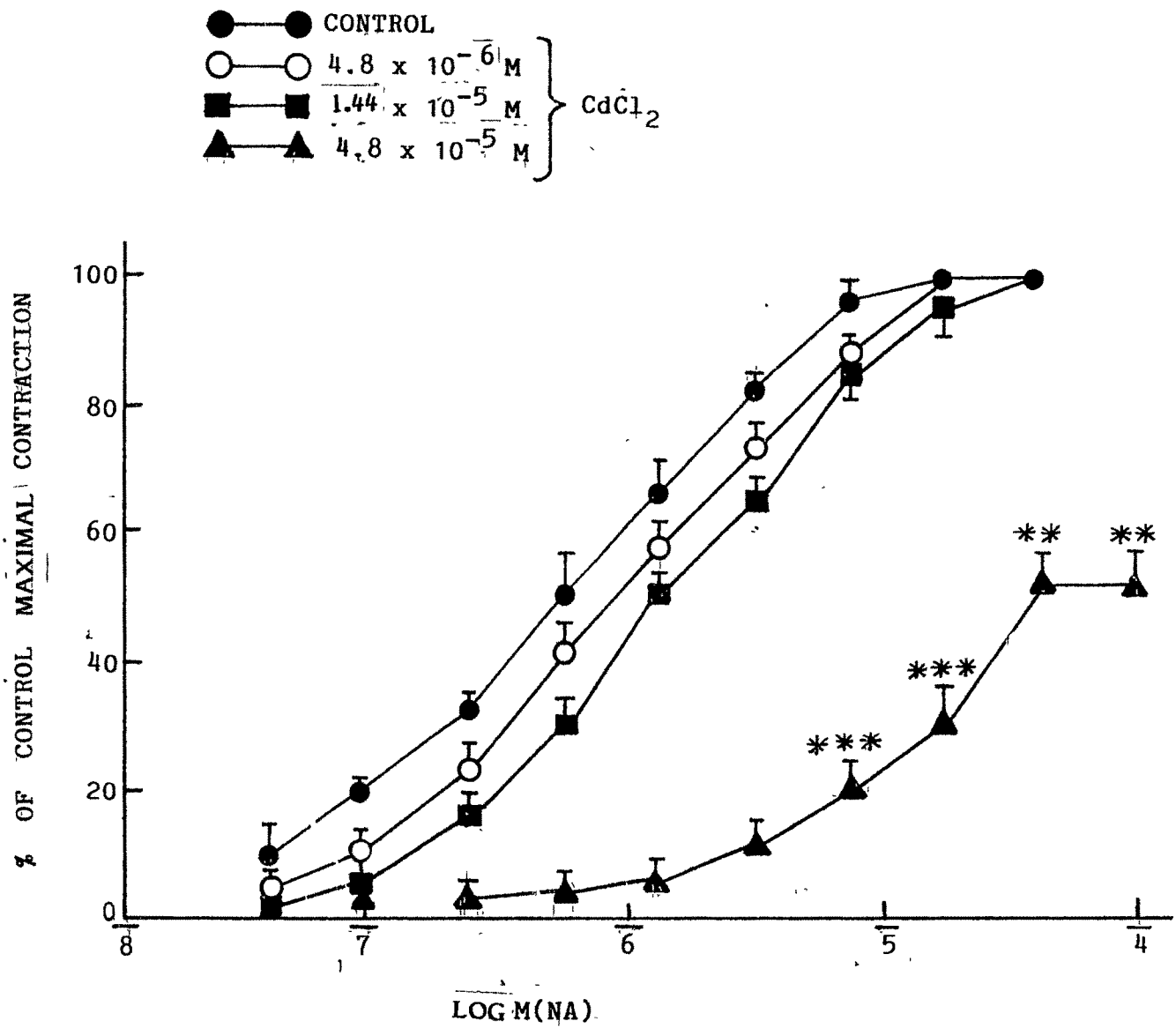

CONTROL
4.8 x 10⁻⁶ M
1.44 x 10⁻⁵ M
4.8 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
8
7
6
5
4
LOG M(NA)
**
**
***
***

Fig. 39 : Dose-response curves of NA in rat isolated anococcygeus muscle. Abscissa indicates the log molar concentration of NA and ordinate the % of control maximal contraction. Control (●—●) responses and those in the presence of 4.8×10^{-6}M (○—○), 1.44×10^{-5}M (■—■), 4.8×10^{-5}M (▲—▲) $CdCl_2$ elicited in reduced calcium (0.63 mM) concentration in the medium. Vertical lines represent SEM (n=5 for each observation; *** $P < 0.001$ as compared with the corresponding control responses).

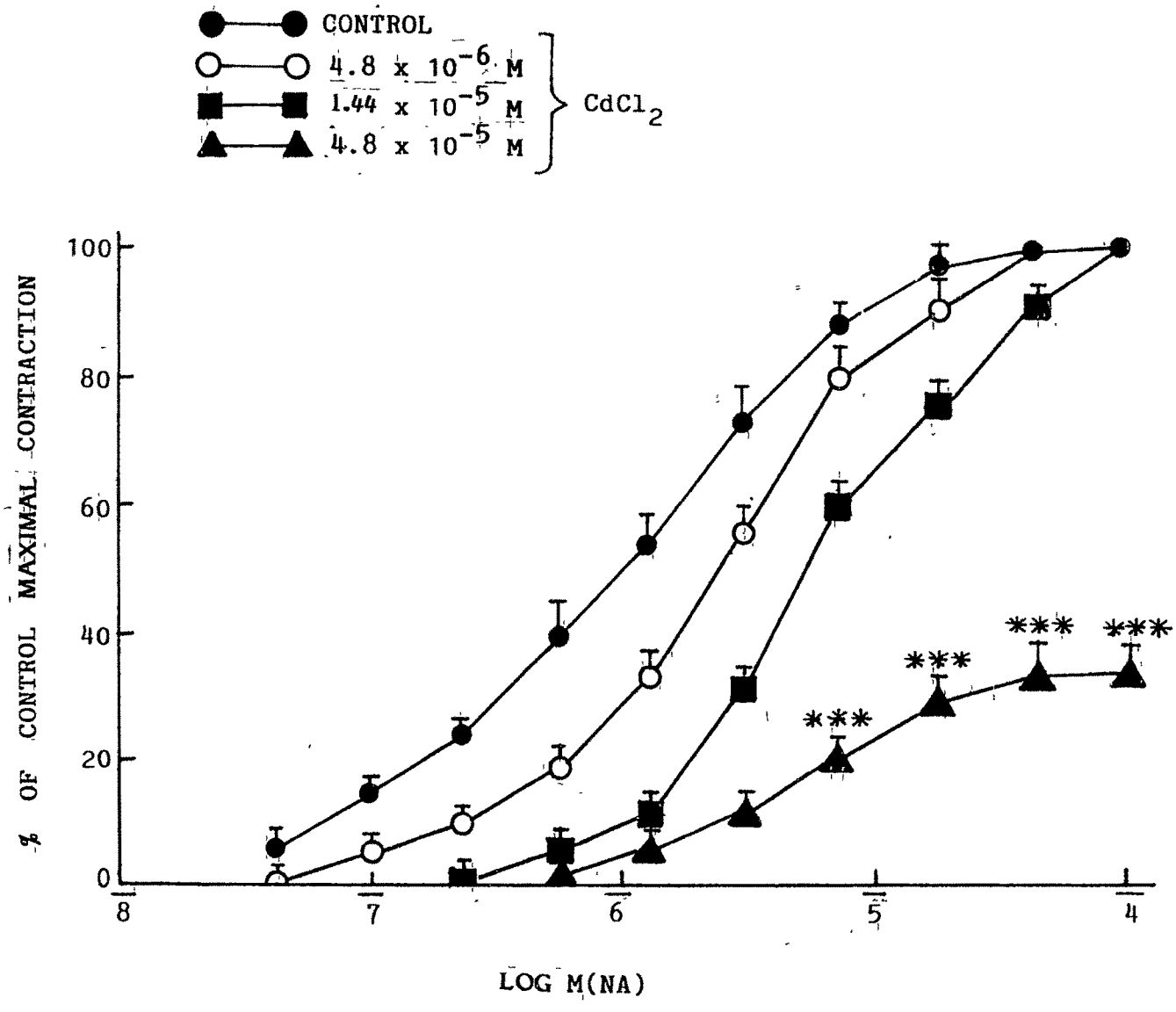

CONTROL
4.8 x 10⁻⁶ M
1.44 x 10⁻⁵ M
4.8 x 10⁻⁵ M
CdCl2
% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
8
7
6
5
4
LOG M(NA)
***
***
***
***

Fig. 40 : The basal perfusion pressure (mm Hg) of hindquarter preparation of control and $CdCl_2$ treated rats. White histogram indicates control pressure. Vertically hatched, stippled and diagonally hatched histograms indicate the pressure of 0.1 mg/kg/day, i.p., for two weeks, 0.5 mg/kg/day, i.p., for two weeks and 1 mg/kg/day, i.p., for two weeks of $CdCl_2$ treated respectively. Vertical lines on the histograms represent SEM (n=4 for each observation; * $P < 0.05$ as compared with the corresponding control).

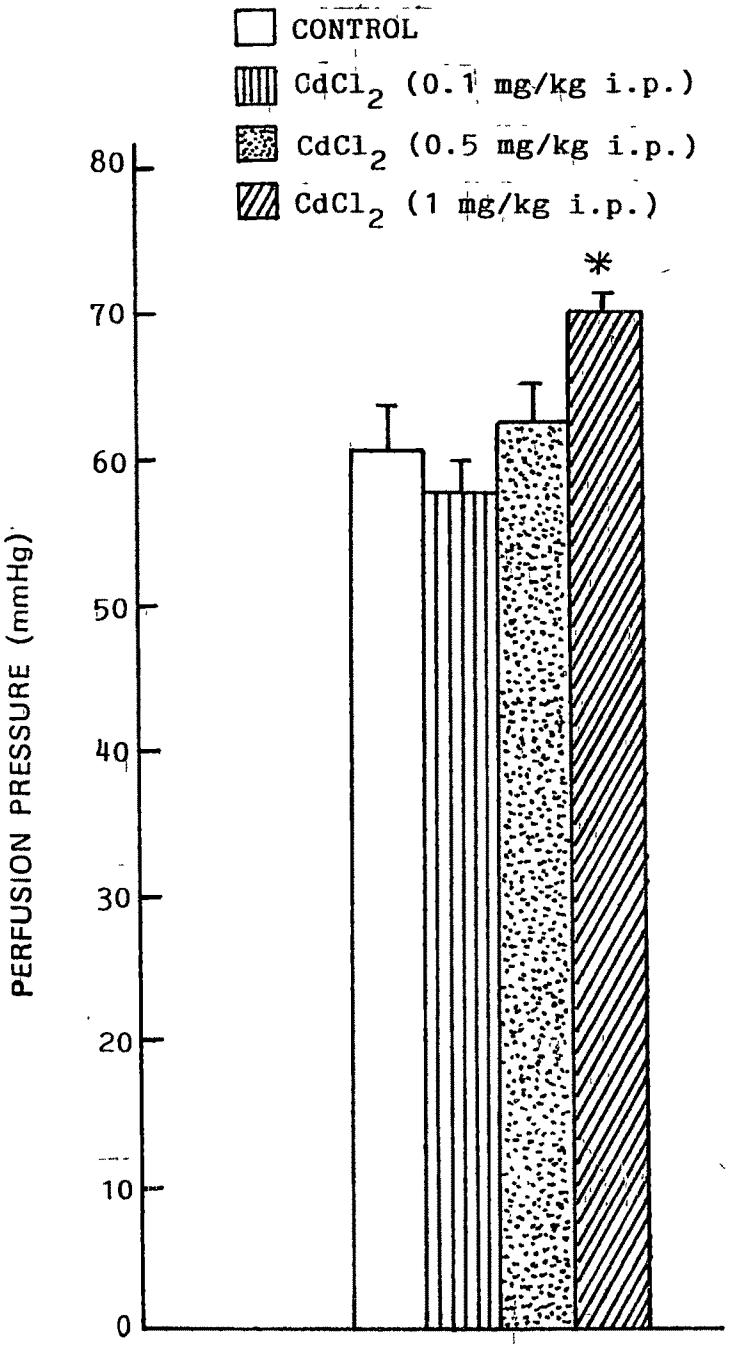
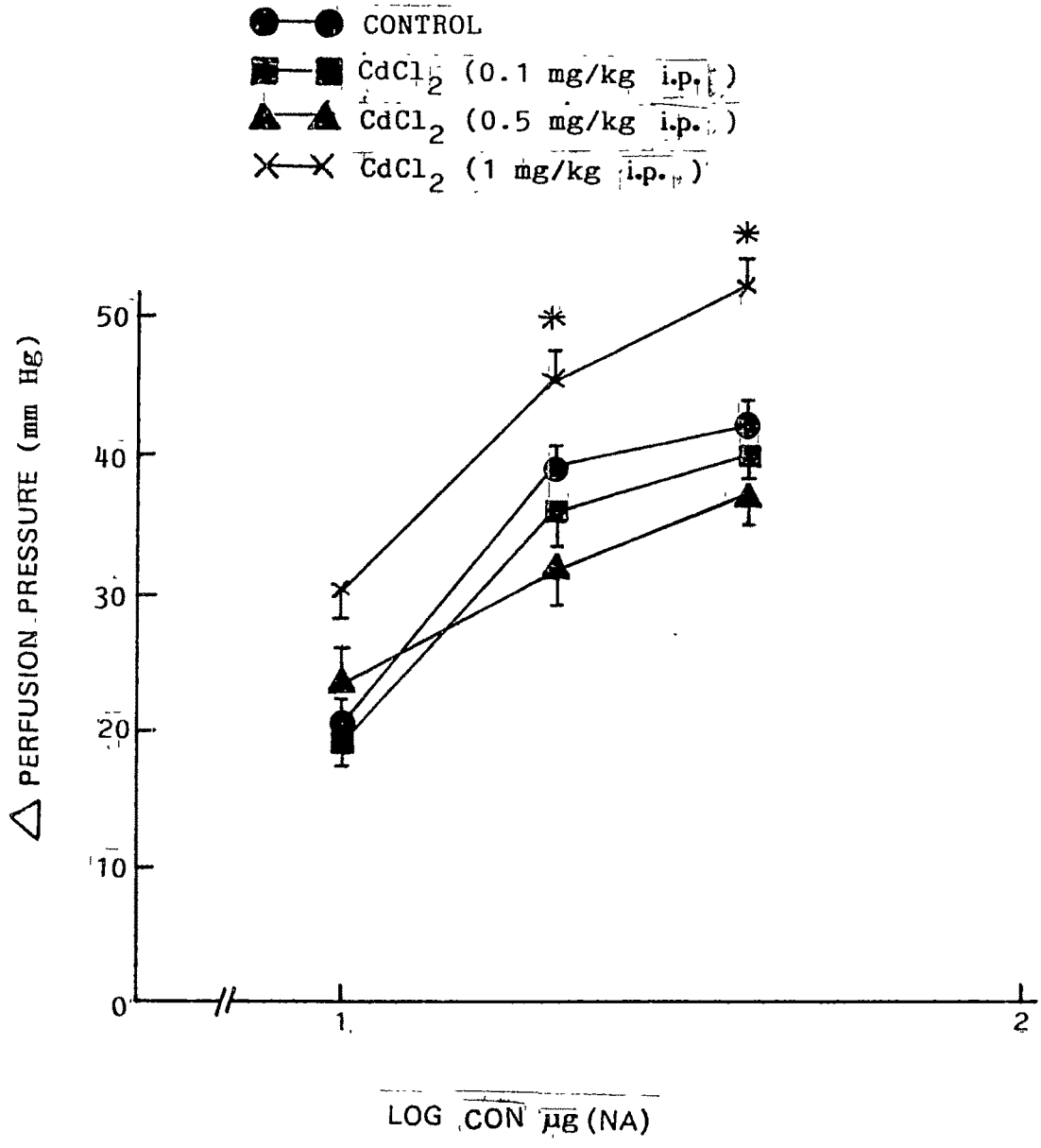

CONTROL
CdCl2 (0.1 mg/kg i.p.)
CdCl2 (0.5 mg/kg i.p.)
CdCl2 (1 mg/kg i.p.)
PERFUSION PRESSURE (mmHg)
80
70
60
50
40
30
20
10
0
*

Fig. 41 : Mean increase in the perfusion pressure (mm Hg) by the intra-arterial administration of NA in the hindquarter préparations. (●—●) indicates control and (■—■), (▲—▲) and (×—×) indicate 0.1 mg/kg,i.p., 0.5 mg/kg, i.p., and 1 mg/kg, i.p. doses of $CdCl_2$ administered daily chronically for two weeks respectively. Abscissa indicates the log concentration of NA and ordinate the change in perfusion pressure (mm Hg). Vertical lines represent SEM (n=4 for each observation; * $P < 0.05$ as compared with the control).


CONTROL
CdCl2 (0.1 mg/kg i.p.)
CdCl2 (0.5 mg/kg i.p.)
CdCl2 (1 mg/kg i.p.)
△ PERFUSION PRESSURE (mm Hg)
50
40
30
20
10
0
1
2
LOG CON µg (NA)

4.2.2.2. IN VITRO SENSITIVITY OF ISOLATED AORTA, PORTAL MESENTERIC VEIN, VAS DEFERENS, AND ANOCOCCYGEUS MUSCLE OBTAINED FROM RATS CHRONICALLY TREATED WITH $CdCl_2$ (1 mg/kg/day, i.p., two weeks) :

The pD_2 value of KCl was not significantly changed in isolated aorta of rats treated with $CdCl_2$ (1 mg/kg, i.p., for two weeks) (Table XIV; Fig. 42)

The pD_2 value of NA was significantly ($P < 0.01$) higher in the isolated aorta of the rats chronically treated with $CdCl_2$ (Table XIV; Fig. 43).

The pD_2 values of KCl and NA (XIV) were not significantly changed in the isolated portal vein, vas deferens and anococcygeus of rats treated chronically with $CdCl_2$ (Fig. 44, 45, 46, 47, 48 and 49).

4.3. BODY WEIGHT :

There was a significant ($P < 0.05$) reduction in the body weight of the animals treated chronically with $CdCl_2$ (0.5 and 1 mg/kg, i.p., two weeks). However, chronic administration of a lower dose of $CdCl_2$ (0.1 mg/kg, i.p., for two weeks) did not produce any significant change in the body weight (Table XV).

Table XIV : pD_2 values of KCl and NA obtained with rat isolated aorta, portal mesentric vein, vas deferens and anococcygeus muscle of control and $CdCl_2$ (1 mg/kg/day, i.p., two weeks) treated rats (n=4 to 5)

Tissues	Agonist	Mean pD_2 value (±SEM)		P value
		Control	$CdCl_2$ treated rats	
(A) Rat aortic strip				
	KCl	1.48±0.05	1.54±0.06	>0.05
	NA	6.90±0.02	7.17±0.05*	<0.01
(B) Rat portal mesentric vein				
	KCl	1.56±0.03	1.58±0.04	> 0.05
	NA	6.20±0.11	6.35±0.07	> 0.05
(C) Rat vas deferens				
	KCl	1.51±0.04	1.54±0.06	>0.05
	NA	5.69±0.04	5.70±0.03	>0.05
(D) Rat anococcy-geus muscle				
	KCl	1.49±0.04	1.42±0.04	>0.05
	NA	6.19±0.09	6.30±0.05	>0.05

Fig. 42 : Dose-response curves of KCl in rat isolated aorta. Abscissa indicates the log molar concentration of KCl and ordinate the % of maximal contraction. Responses of control aorta (●—●) and those from animals chronically treated with $CdCl_2$ (1 mg/kg/day, i.p., for two weeks) (▲—▲) are shown. Vertical lines represent SEM (n=5 for each observation).

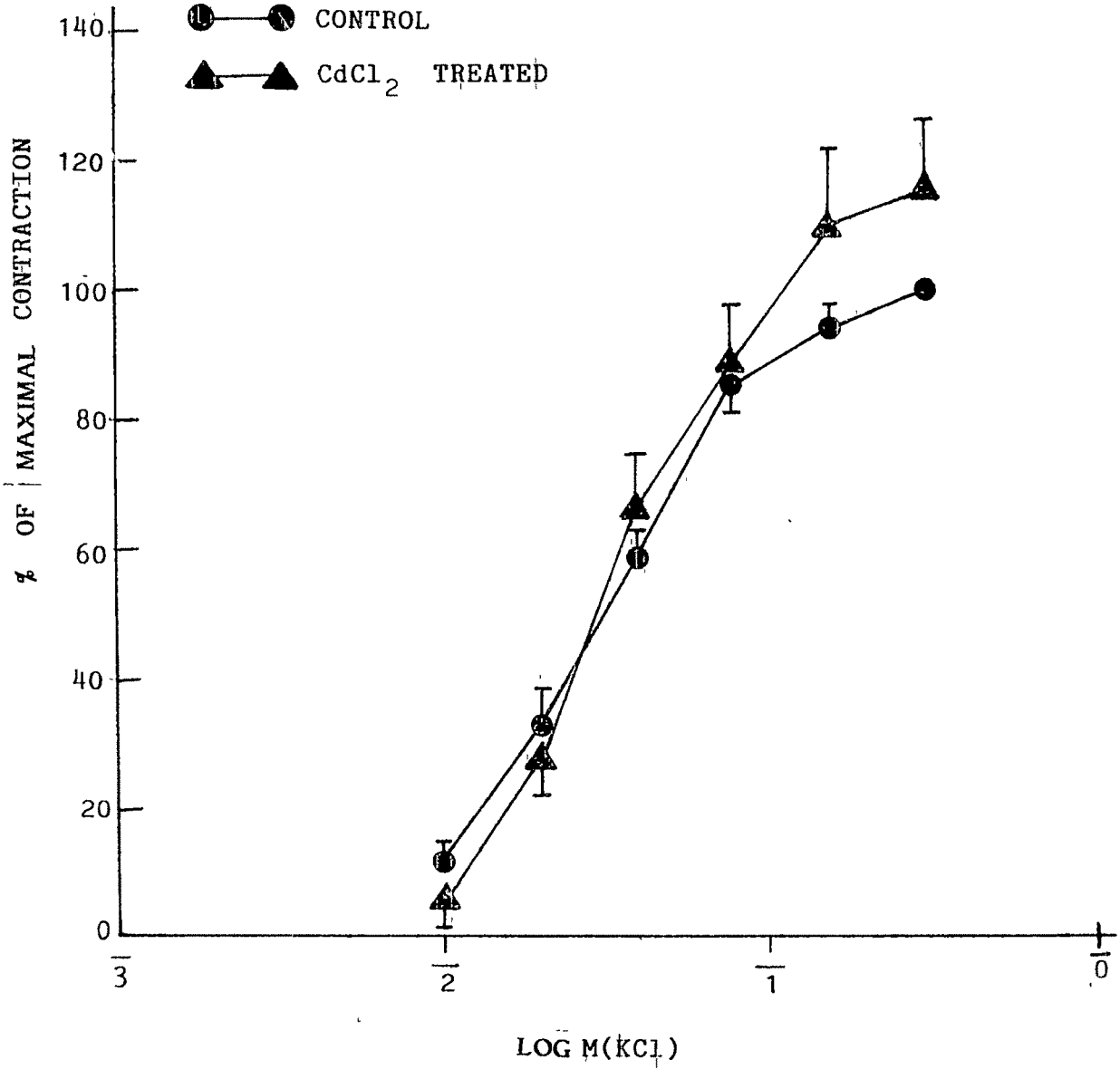

CONTROL
CdCl2 TREATED
% OF MAXIMAL CONTRACTION
140
120
100
80
60
40
20
0
3
2
1
0
LOG M(KCl)

Fig. 43 : Dose-response curves of NA in rat isolated aorta. Abscissa indicates the log molar concentration of NA and ordinate the % of maximal contraction. Response of control aorta (●—●) and those from animals chronically treated with $CdCl_2$ (1 mg/kg/day, i.p., for two weeks)(▲—▲) are shown. Vertical lines represent SEM (n=5 for each observation. * $P<0.05$ as compared to the corresponding control responses).

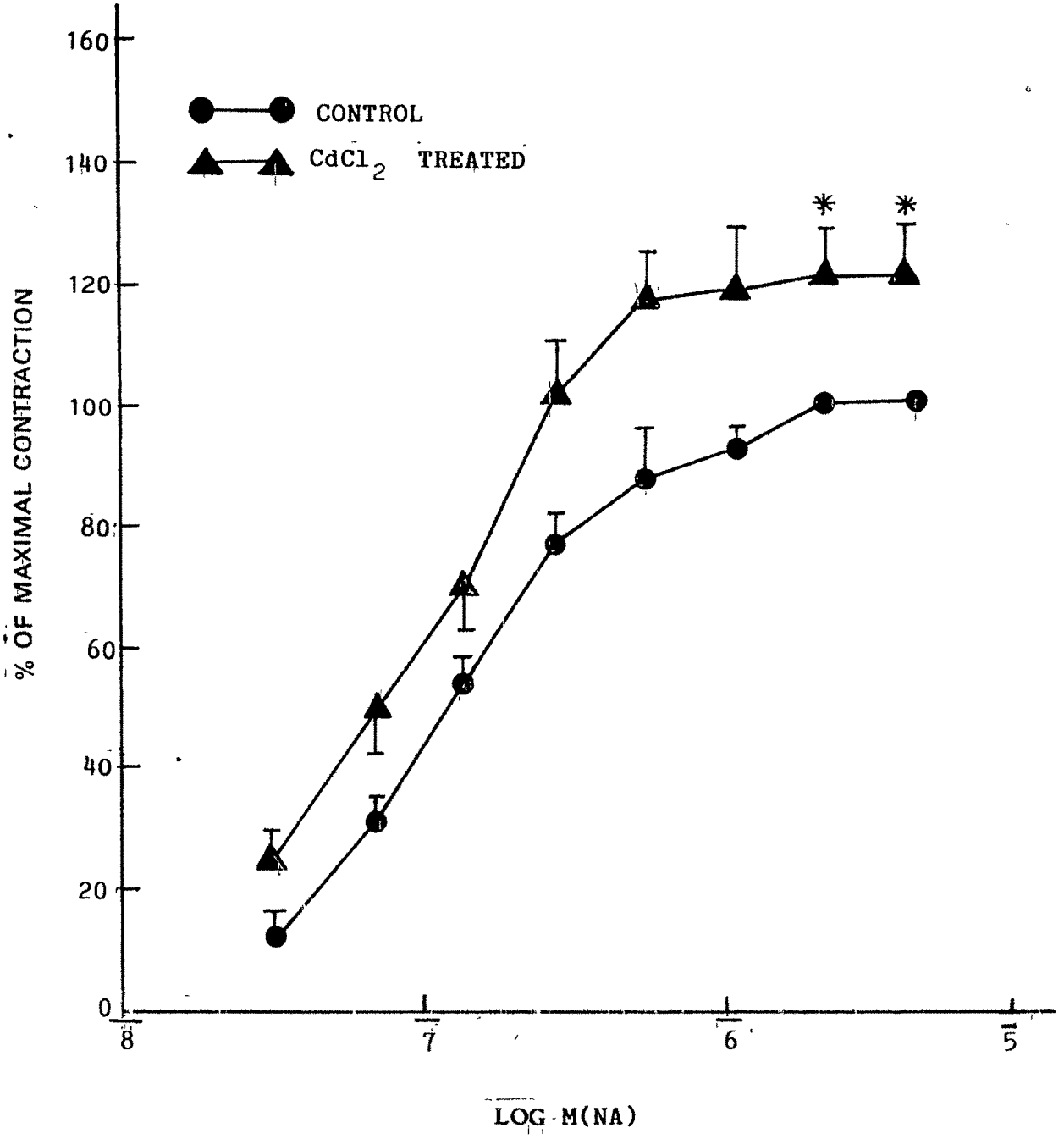

160
140
120
100
80
60
40
20
0
CONTROL
CdCl2 TREATED
% OF MAXIMAL CONTRACTION
*
*
8
7
6
5
LOG M(NA)

Fig. 44 : Dose-response curves of KCl in rat isolated portal mesenteric vein. Abscissa indicates the log molar concentration of KCl and ordinate the % of maximal contraction. Response of control portal vein (●—●) and those from animals chronically treated with $CdCl_2$ (▲—▲) (1 mg/kg/day, i.p., for two weeks) are shown. Vertical lines represent SEM (n=5 for each observation).

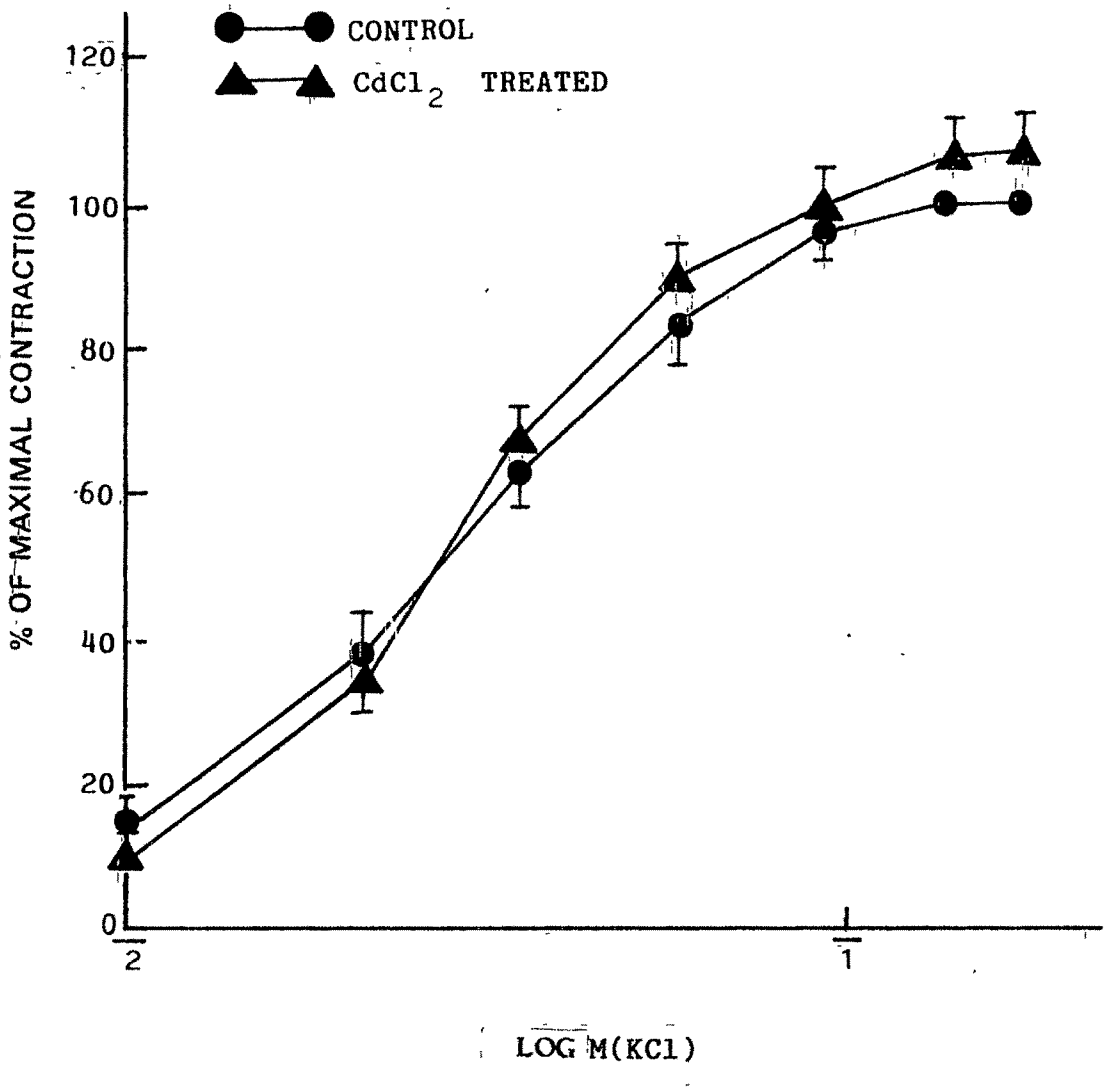

CONTROL
CdCl2 TREATED
% OF MAXIMAL CONTRACTION
120
100
80
60
40
20
0
2
1
LOG M(KCl)

Fig. 45 : Dose-response curves of NA in rat isolated portal vein. Abscissa indicates the log molar concentration of NA and ordinate the % of maximum contraction. Responses of control portal vein (●—●) and those from animals chronically treated with $CdCl_2$ (1 mg/kg/day, i.p., for two weeks) (▲—▲) are shown. Vertical lines represent SEM (n=4 for each observation).

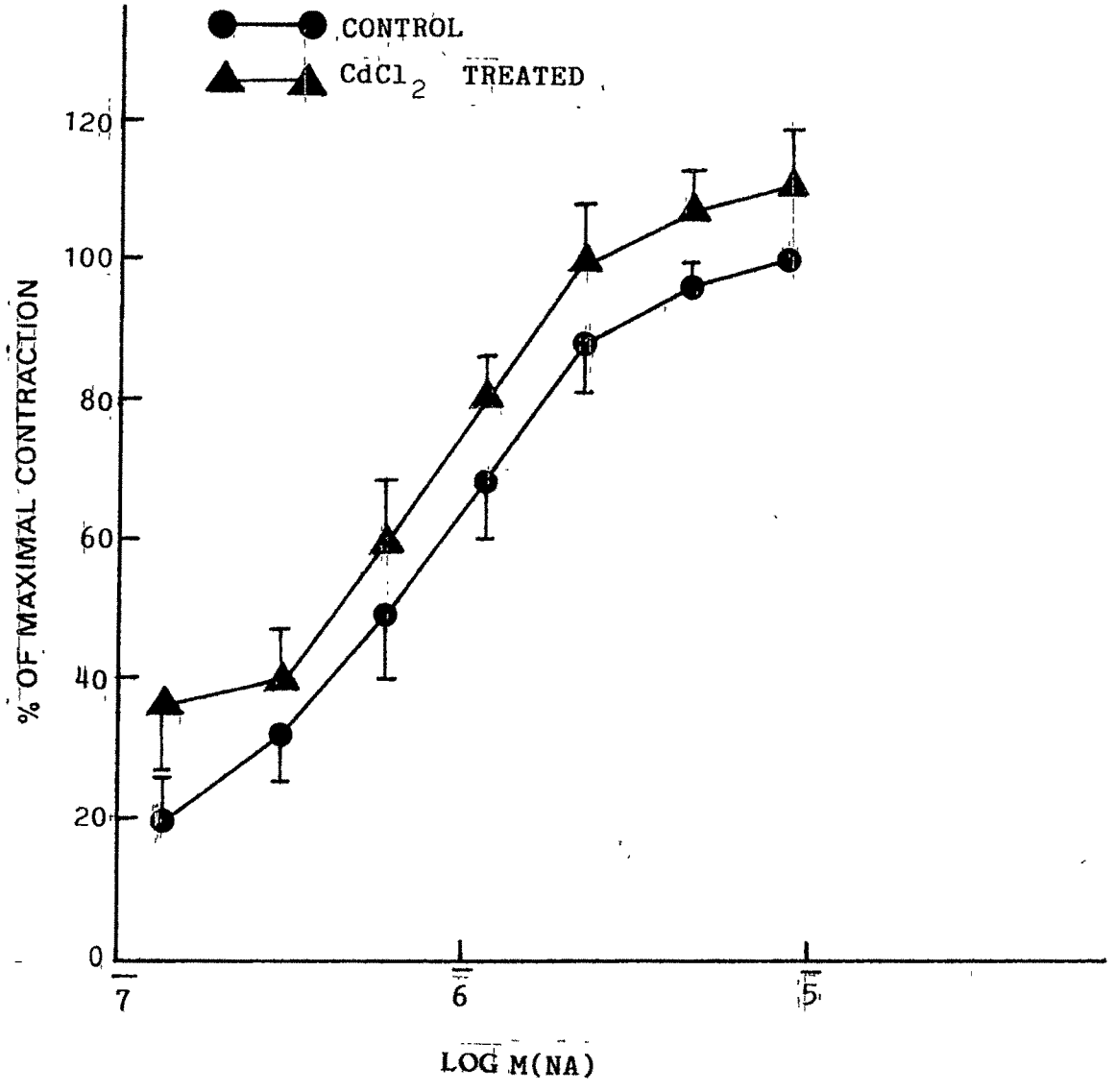

CONTROL
CdCl2 TREATED
120
100
80
60
40
20
0
% OF MAXIMAL CONTRACTION
7
6
5
LOG M(NA)

Fig. 46 : Dose-response curves of KCl in rat isolated vas deferens. Abscissa indicates the log molar concentration of KCl, and ordinate the % of maximal contraction. Responses of control vas deferens (●—●) and those from animals chronically treated with $CdCl_2$ (1 mg/kg/day, i.p., for two weeks) (▲—▲) are shown. Vertical lines represent SEM (n=4 for each observation).

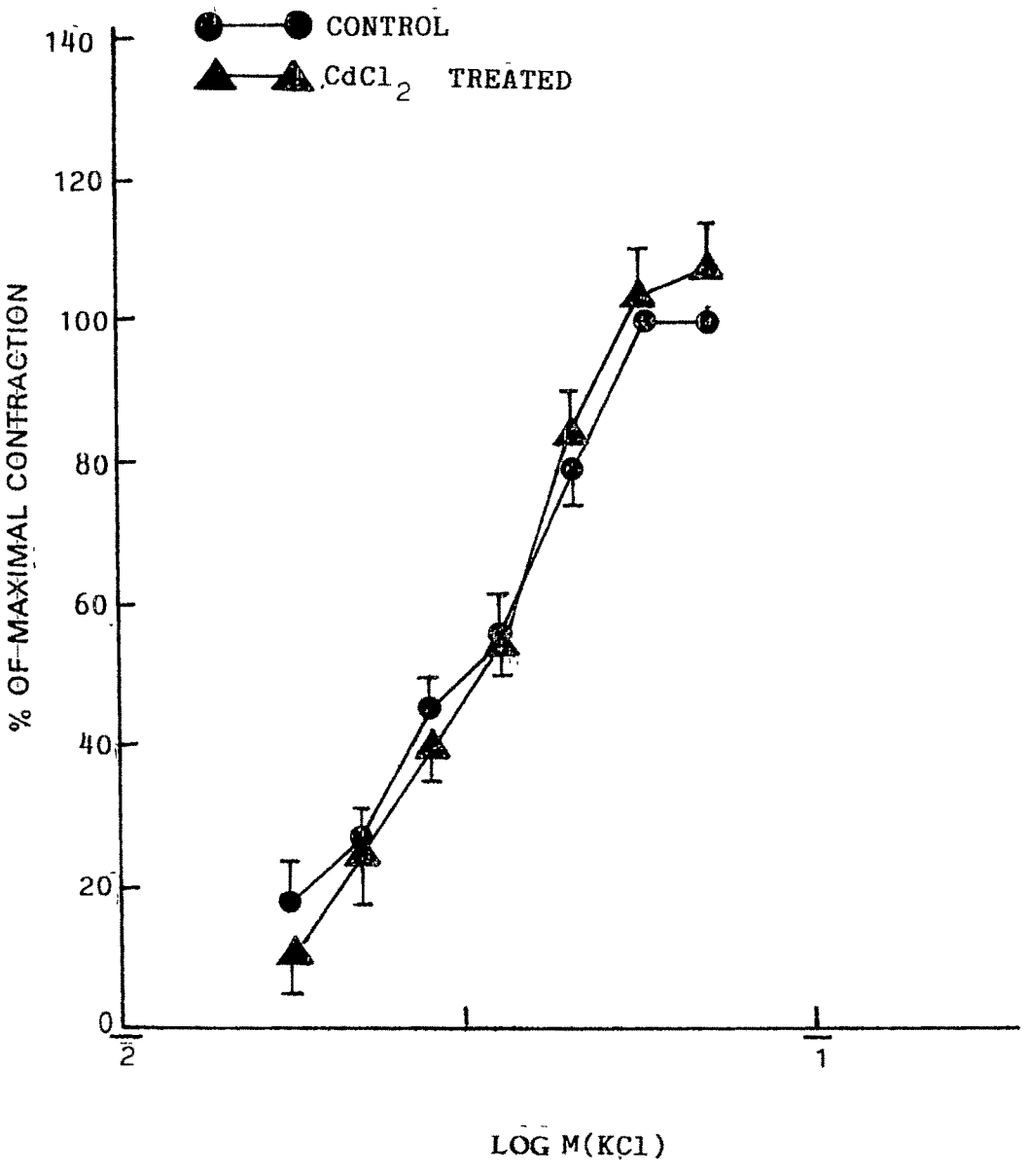

CONTROL
CdCl2 TREATED
140
120
100
80
60
40
20
0
% OF MAXIMAL CONTRACTION
2
1
LOG M(KCl)

Fig. 47 : Dose response curves of NA in rat isolated vas deferens. Abscissa indicates the log molar concentration of NA and ordinate the % of maximal contraction. Responses of control vas deferens (●——●) and those from animals chronically treated with $CdCl_2$ (1 mg/kg/day, i.p., for two weeks) (▲——▲) are shown. Vertical lines represent SEM (n=5 for each observation).

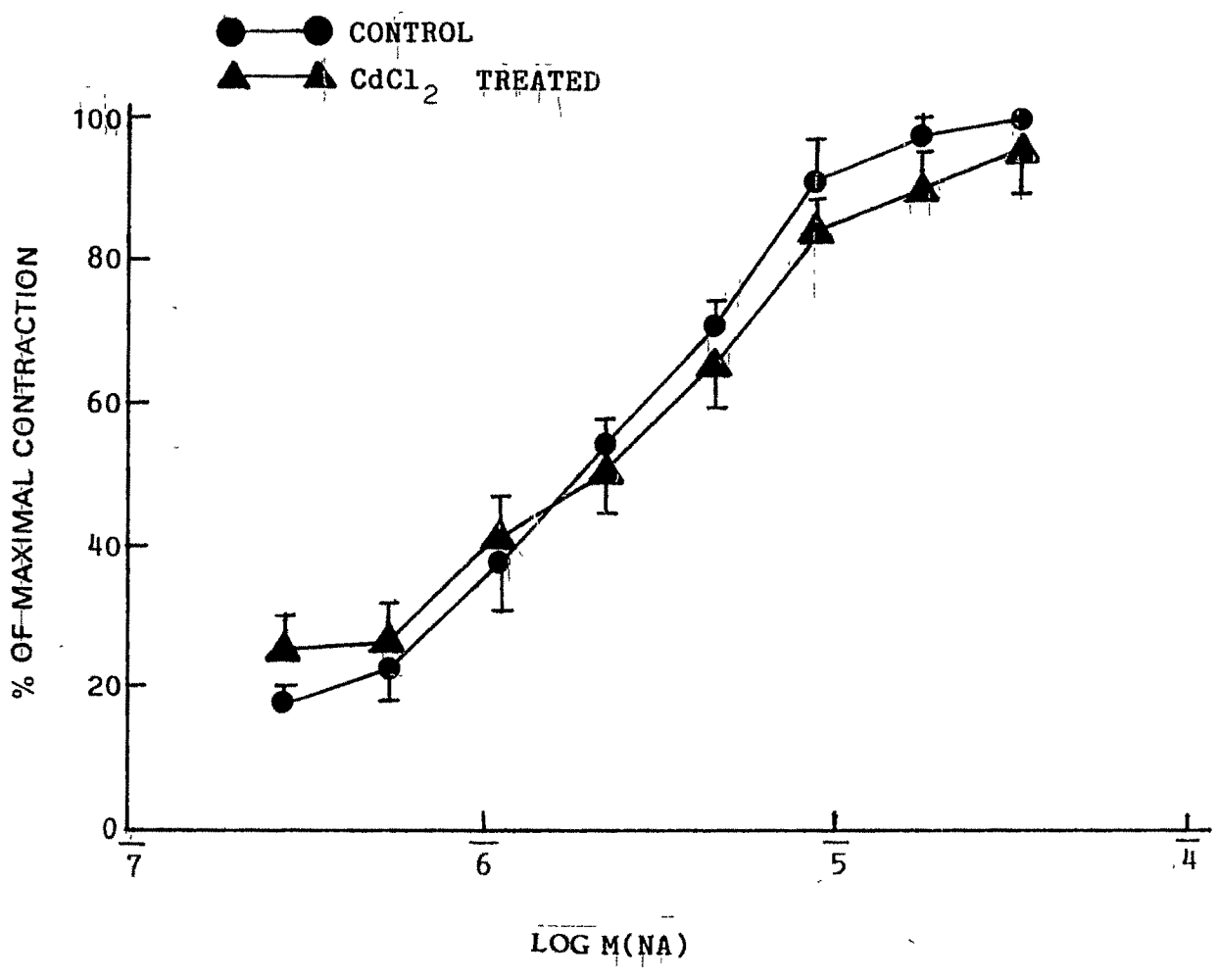

CONTROL
CdCl2 TREATED
% OF MAXIMAL CONTRACTION
100
80
60
40
20
0
7
6
5
4
LOG M(NA)

Fig. 48 : Dose-response curves of KCl in rat isolated anococcygeus muscle. Abscissa indicates the log molar concentration of KCl and ordinate the % of maximal contraction. Responses of control anococcygeus (●—●) and those from animals chronically treated with $CdCl_2$ (1 mg/kg/day, i.p., for two weeks)(▲—▲) are shown. Vertical lines represent SEM (n=4 for each observation).

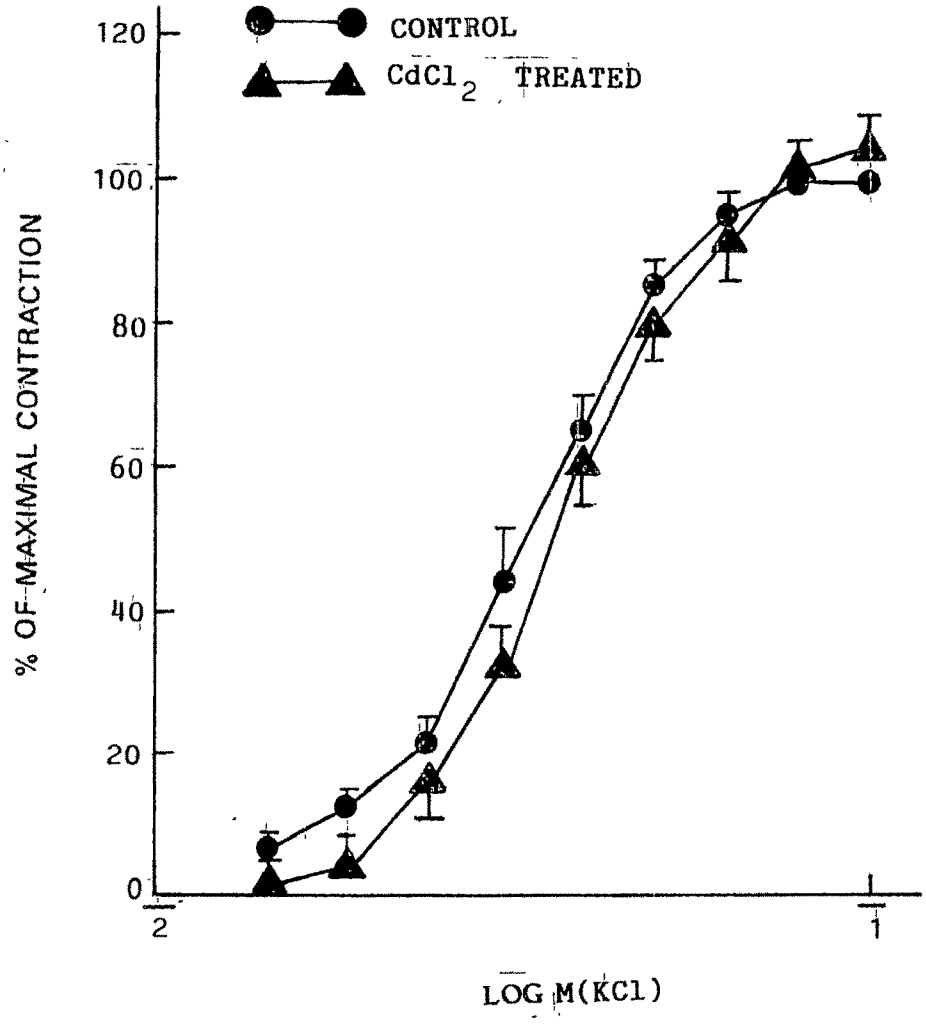

CONTROL
CdCl2 TREATED
120
100
80
60
40
20
0
% OF MAXIMAL CONTRACTION
2
1
LOG M(KCl)

Fig. 49 : Dose-response curves of NA in rat isolated anococcygeus muscle. Abscissa represents the log molar concentration of NA and ordinate the % of maximum contraction. Response of control anococcygeus muscles (●——●) and those from animals chronically treated with $CdCl_2$ (1 mg/kg/day, i.p., for two weeks) (▲——▲) are shown. Vertical lines represent SEM (n=4 for each observation).

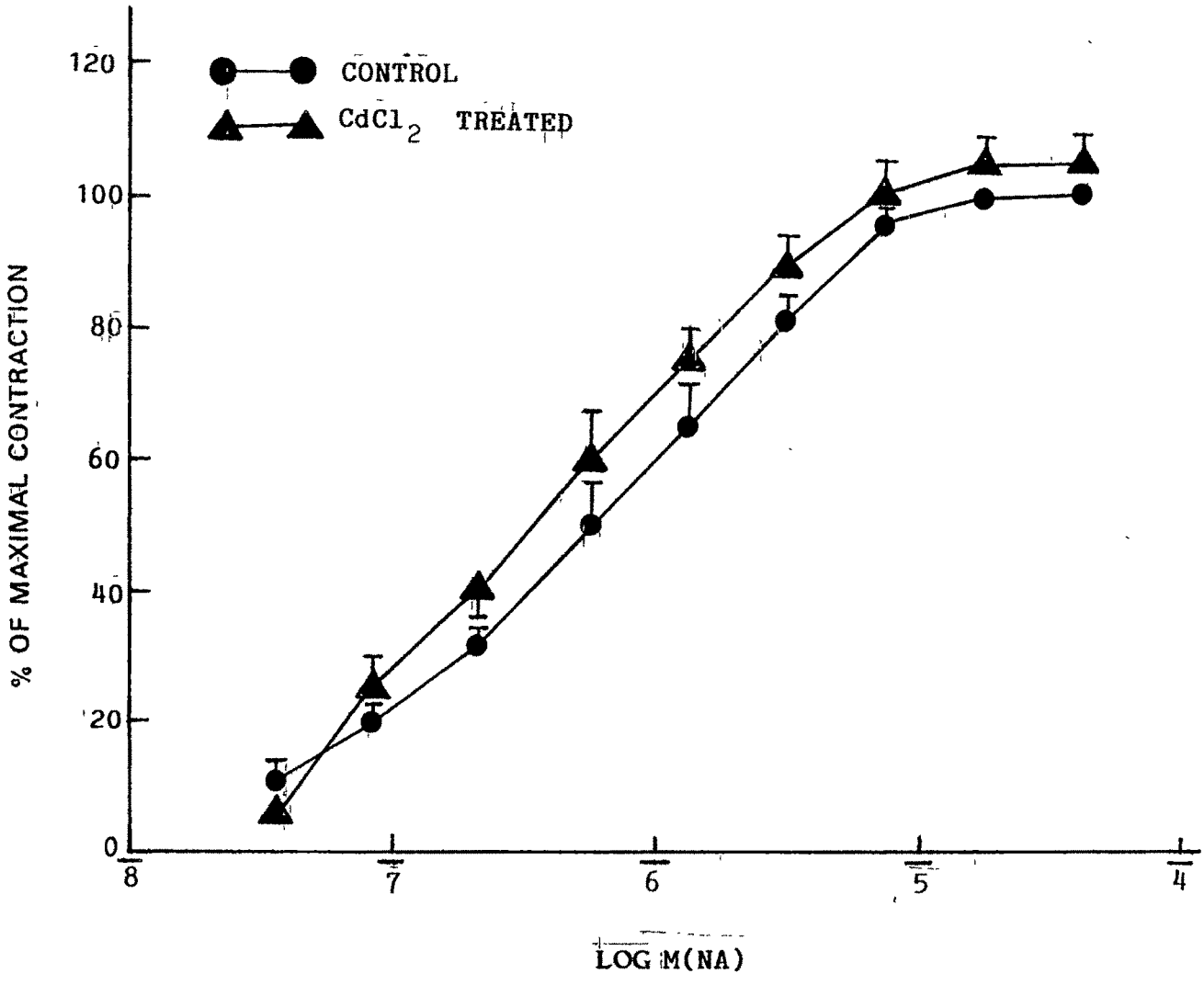

CONTROL
CdCl2 TREATED
% OF MAXIMAL CONTRACTION
120
100
80
60
40
20
0
8
7
6
5
4
LOG M(NA)

Table XV : Effect of 2 week $CdCl_2$ treatment on body weight of female rats (n=6 to 10)

Group	Dose (mg/kg/day)	Body wt. (g)±SEM		P
		Before $CdCl_2$ treatment	At the end of 2 week $CdCl_2$ treatment	
Control	0.2 ml 0.9% NaCl, i.p.	210±64	235±10.1*	<0.05
$CdCl_2$ treated	0.1 i.p.	220±7.3	232± 5.7	>0.05
	0.5 i.p.	252±4.0	231±6.0*	<0.05
	1 i.p.	254±7.7	220± 8.2*	<0.05

4.4. HISTOPATHOLOGICAL OBSERVATIONS :

4.4.1. KIDNEY :

Microscopic examination of the kidney showed cloudy swelling in the renal tubules. At places, there was sparse infiltration with chronic inflammatory cells (Fig. 50A).

4.4.2. HEART :

Slight inflammatory exudate was seen on the serosal surface. Except this, there was no significant change in the heart (Fig. 50B).

4.4.3. LIVER :

There was sparse inflammatory exudate along with increased fibrous tissue in the portal tract. There was also some cloudy swelling in the hepatocytes (Fig. 50 C).

Fig. 50 : Histopathological sections of kidney, heart and liver of normal (left) and $CdCl_2$ (1 mg/kg/day, i.p., for two weeks) treated (right) rats. Magnification 80X.

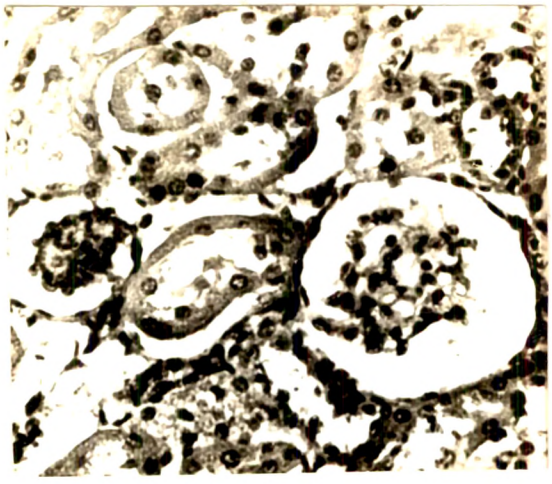
(A) Treated kidney section shows cloudy swelling (shown by arrow) in the renal tubules.

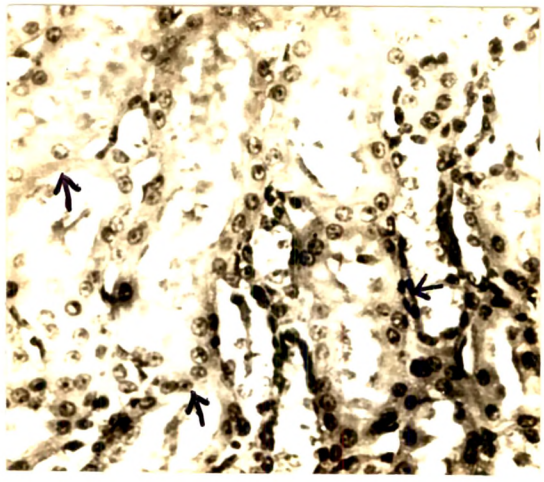
(B) Treated heart section shows slight inflammatory exudate on the serosal surface (shown by arrow).

(C) Treated liver section shows slight inflammatory exudate along with increased fibrous tissue in the portal tract (shown by arrow). Cloudy swelling is also seen in the hepatocyte (shown by the letter 'C').

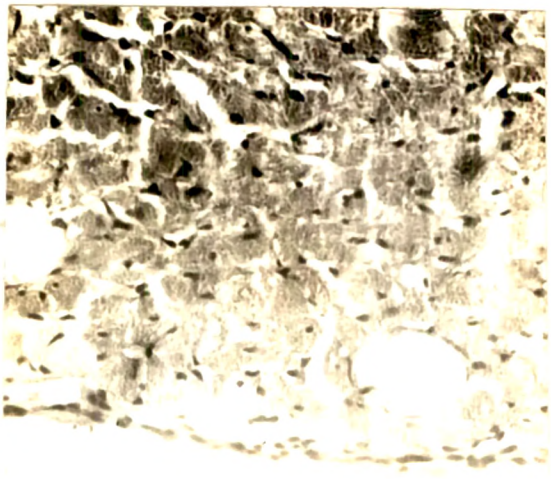
No apparent abnormalities are visible in the normal (control) rats.

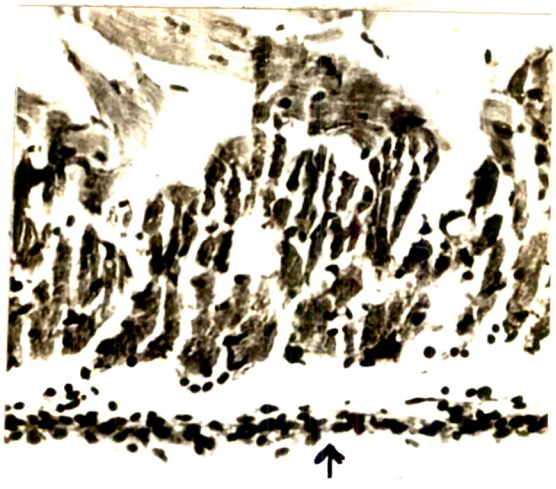
A





B





C