III. RESULTS

Results - Part 1

In vivo experiments

A. Stimulants (Agonists)

1. Acetylcholine (Ach)

Ach, injected into the carotid artery of dogs in doses ranging from 5 to 100 µg/kg body weight elicited a dose-dependent release of ADH (Fig. 6). The elevated hormone level was reflected in reduced urine flow, resulting in antidiuresis, however, there was no change in plasma osmolality with any of the doses of Ach (Fig. 7).

2. Nicotine

Nicotine at a dose range of 5 to 100 µg/kg body weight, like Ach, elicited a dose dependent release of ADH (Fig. 8). In this group of dogs the mean arterial blood pressure (MABP) was markedly elevated by nicotine. This increase was proportional to the dose of nicotine. The urine output was reduced with each dose of nicotine, however, the plasma osmolality remained constant (Fig. 9).

3. McN-A-343-11

McN-A-343-11, a muscuranic ganglion stimulant drug, when injected into the carotid artery in a dose range of 100 to 1600 µg/kg body weight, in contrast to nicotine and Ach, did not produce a dose-dependent change in the plasma level of ADH (Fig. 10). The urine flow as well as the plasma osmolality did not change significantly. The

Figure 6

Dose-response curve of Ach-induced ADH release, in dogs. Each point of the curve represents the mean of 5 observations ± S.E.M.

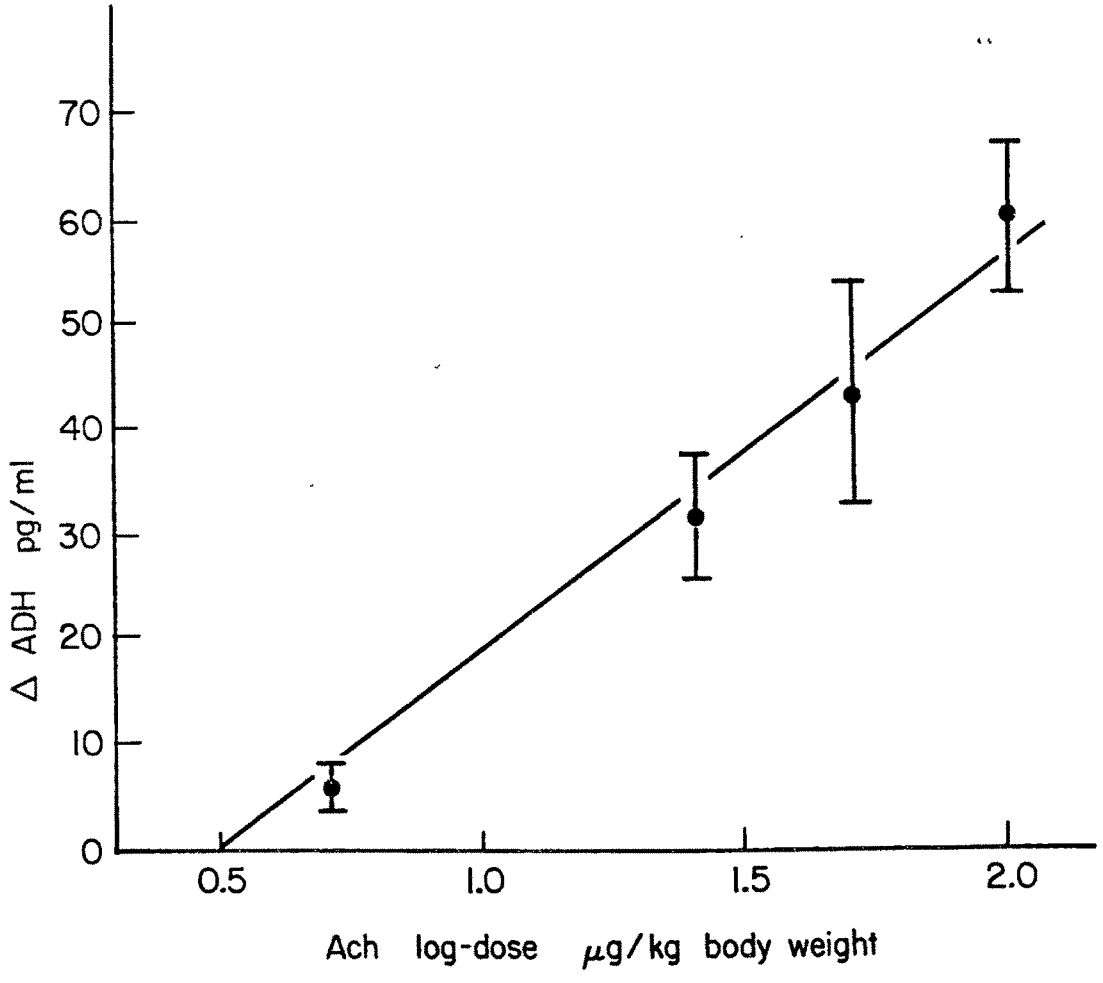

70
60
50
40
30
20
10
0
Δ ADH pg/ml
0.5
1.0
1.5
2.0
Ach log-dose μg/kg body weight

Figure 7

Effect of different doses of Ach on urine flow and plasma osmolality. Each point represents the mean of 5 observations ± S.E.M.

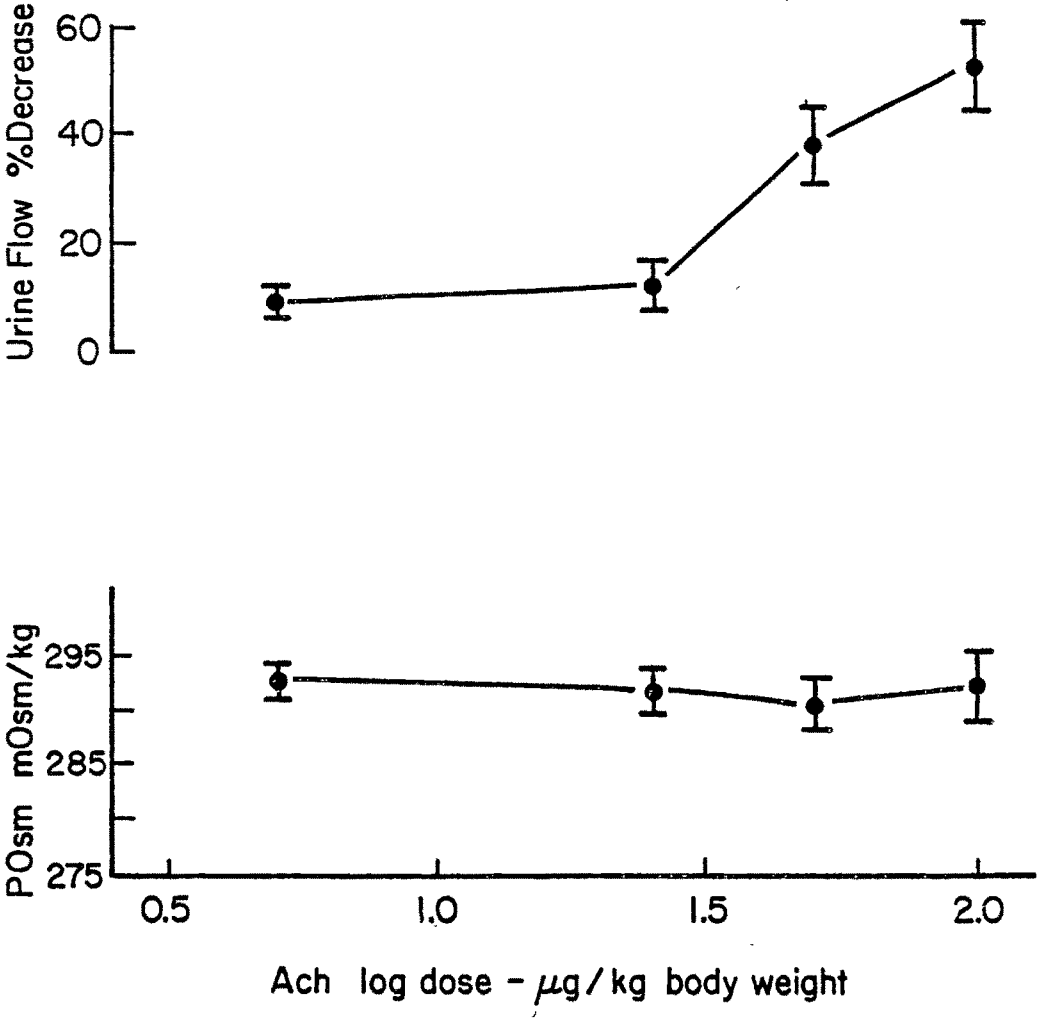

Urine Flow %Decrease
60
40
20
0
POsm mOsm/kg
295
285
275
0.5
1.0
1.5
2.0
Ach log dose - μg / kg body weight

Figure 8

Dose-response curve of nicotine-induced ADH release. Each point represents the mean of 6 observations ± S.E.M.

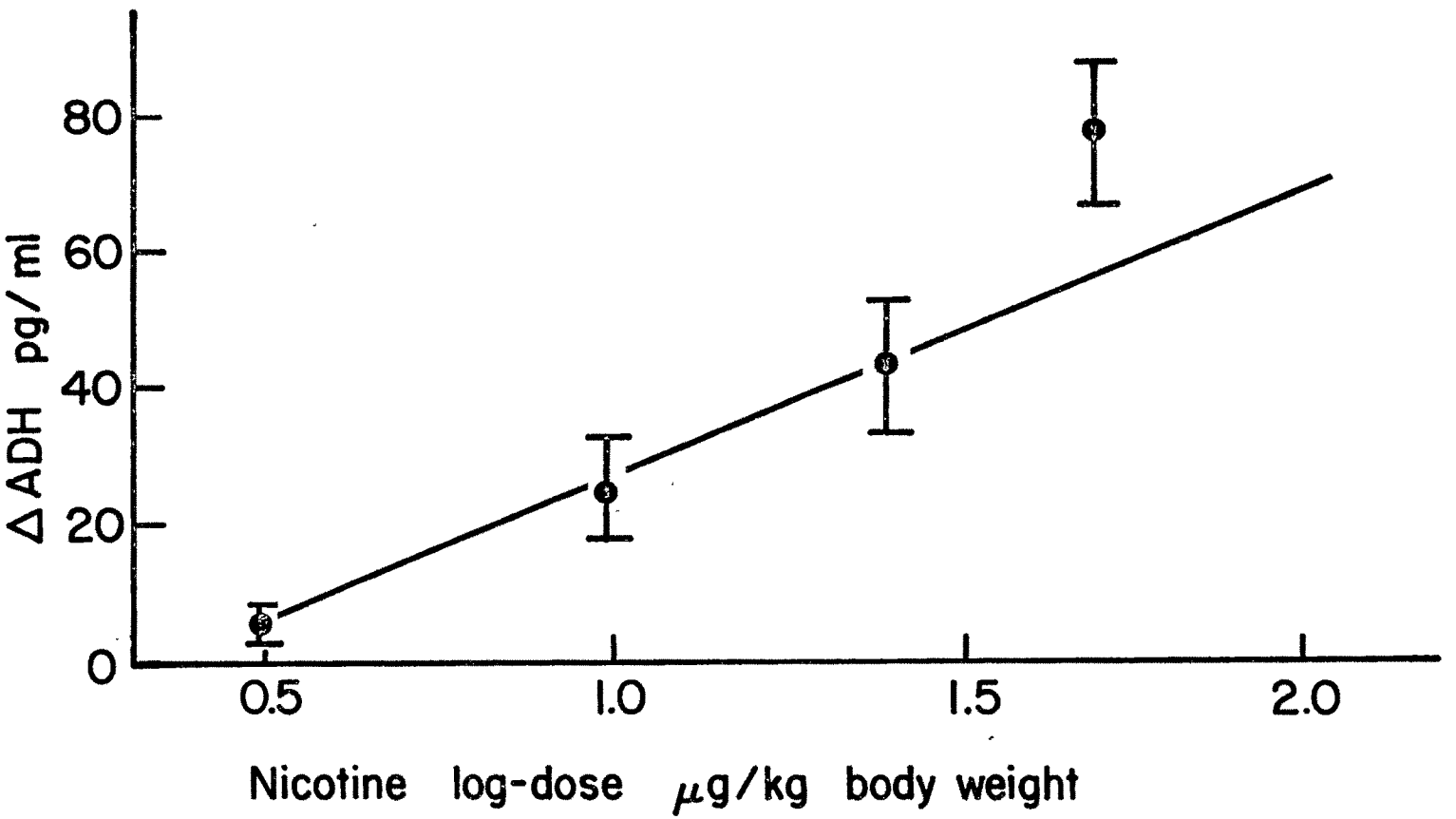

80
60
40
20
0
ΔADH pg/ml
0.5
1.0
1.5
2.0
Nicotine log-dose μg/kg body weight

Figure 9

Effect of varying doses of nictoine on the mean arterial blood pressure, urine flow and plasma osmolality of the dog. Each point represents the mean of 6 observations ± S.E.M.

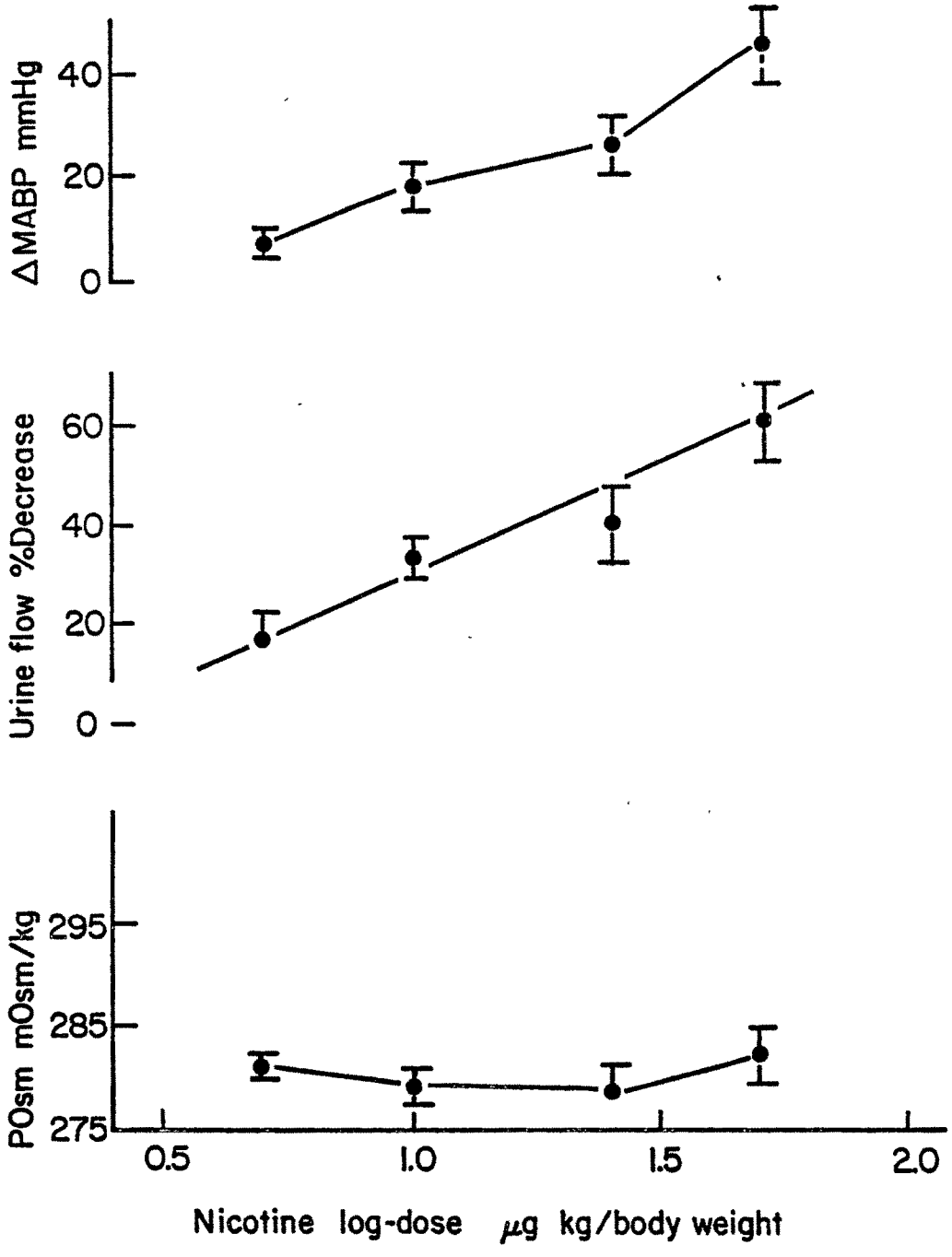

ΔMABP mmHg
40
20
0
Urine flow %Decrease
60
40
20
0
POsm mOsm/kg
295
285
275
0.5
1.0
1.5
2.0
Nicotine log-dose μg kg/body weight

Figure 10

Dose-response curve of McN-A-343-11-induced ADH release in dogs. Each point represents the mean of 5 observations ± S.E.M.

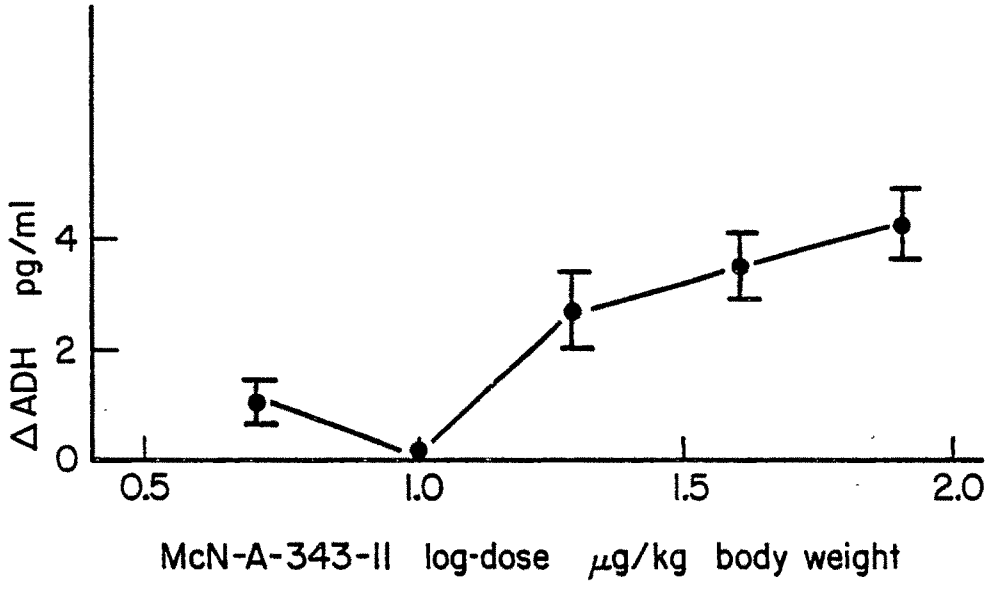
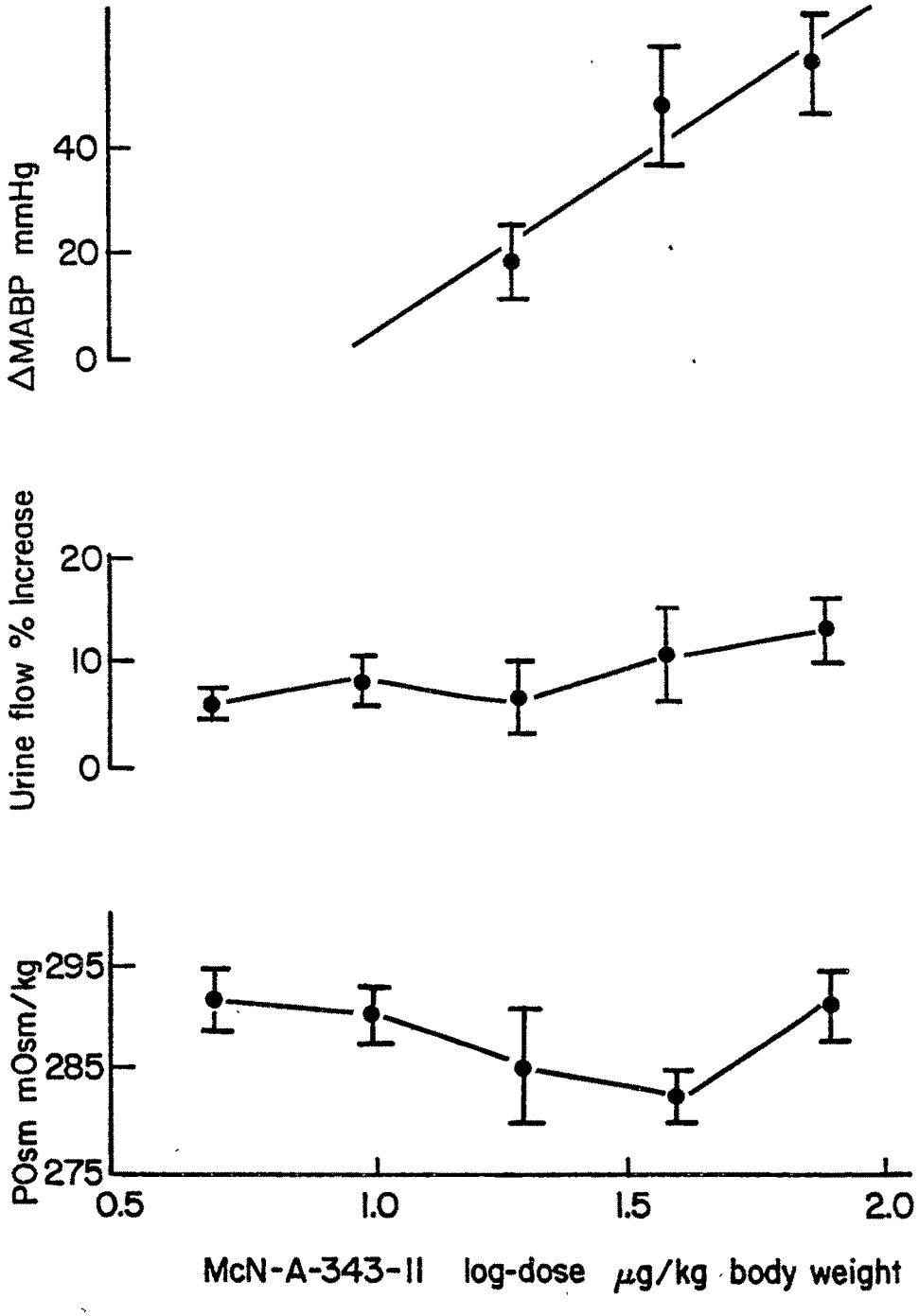

ΔADH pg/ml
0
2
4
0.5
1.0
1.5
2.0
McN-A-343-11 log-dose μg/kg body weight

Figure 11

Effect of varying doses of McN-A-343-11, in mean arterial blood pressure, urine flow and plasma osmolality of dogs. Each point is the mean of 5 observations ± S.E.M.


ΔMABP mmHg
40
20
0
Urine flow % Increase
20
10
0
POsm mOsm/kg
295
285
275
0.5
1.0
1.5
2.0
McN-A-343-II log-dose μg/kg body weight

MABP was unchanged with low doses but elevated with higher doses, again probably as a result of stimulation of sympathetic ganglia (Fig. 11).

4. Angiotensin (A-II)

A-II, like McN-A-343-II in a dose range of 1 to 160 ng/kg body weight did not elicit a dose-dependent increase in the plasma ADH level (Fig. 12). With 5 and 10 ng/kg, A-II produced a small (not significant) increase in ADH level (0.05 to 3.2 pg/ml); but it was not dose-related. There was an increase in MABP corresponding with the dose injected. The urine flow as well as the plasma osmolality were, however, unchanged (Fig. 13).

5. Noradrenaline (NA)

NA when injected into the carotid artery in a dose range of 5 to 50 µg/kg body weight elicited a dose-related ADH release (Fig. 14). However, this increase in ADH release was not reflected in urine output as expected. Instead there was a diuresis. The plasma osmolality was unchanged. The MABP followed a pattern similar to that of A-II (Fig. 15).

B. Antagonists

1. Mecamylamine

NaCl (3 M) was infused into the carotid artery of dogs. This hyperosmotic stimulation increased the ADH level by 12.14±0.08 pg/ml (mean ± SE) and when the same infusion was repeated after mecamylamine (3 to 5 mg/kg) the increase in ADH level was only 2.86±1.51 pg/ml.

Figure 12

Dose-response curve of A-II-induced ADH release in dogs. Each point is the mean of 4 observations ± S.E.M.

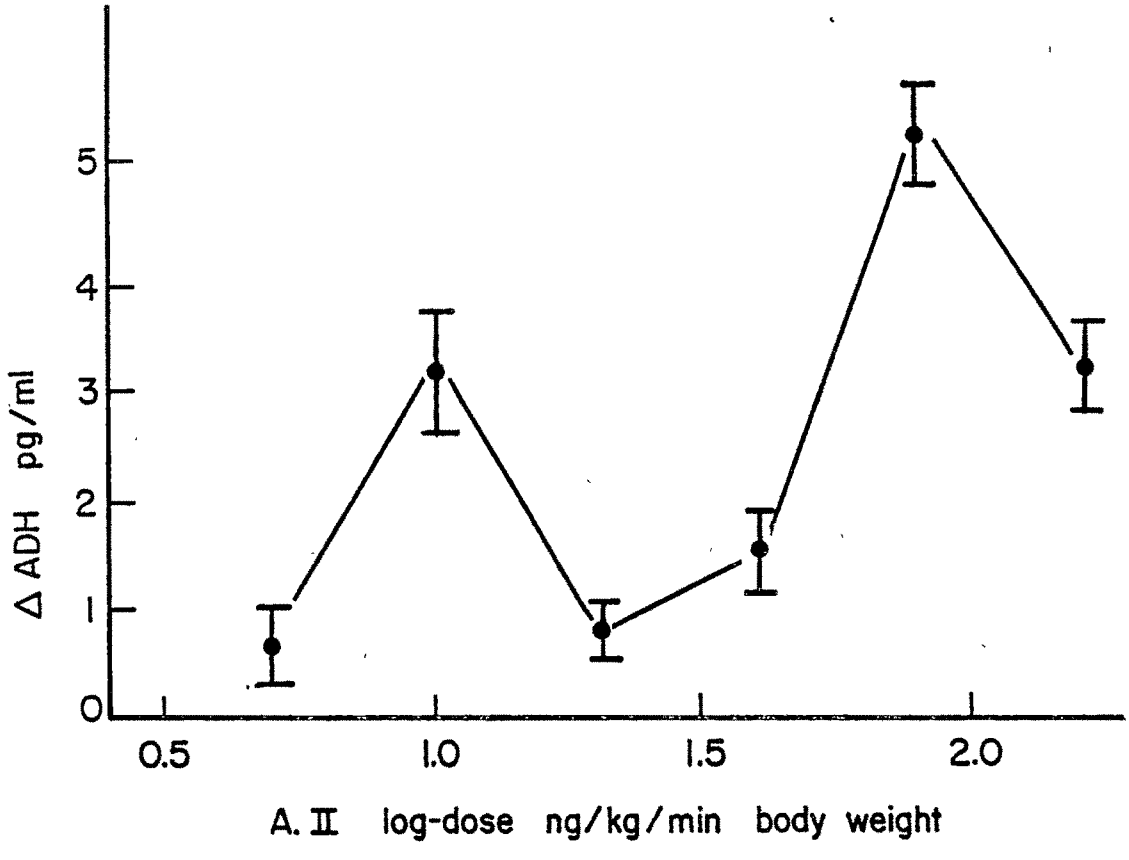

Δ ADH pg/ml
0
1
2
3
4
5
0.5
1.0
1.5
2.0
A. II log-dose ng/kg/min body weight

Figure 13

Changes in mean arterial pressure, urine flow and plasma osmolality of dogs induced by angiotensin-II. Each point is the mean of 4 observations ± S.E.M.

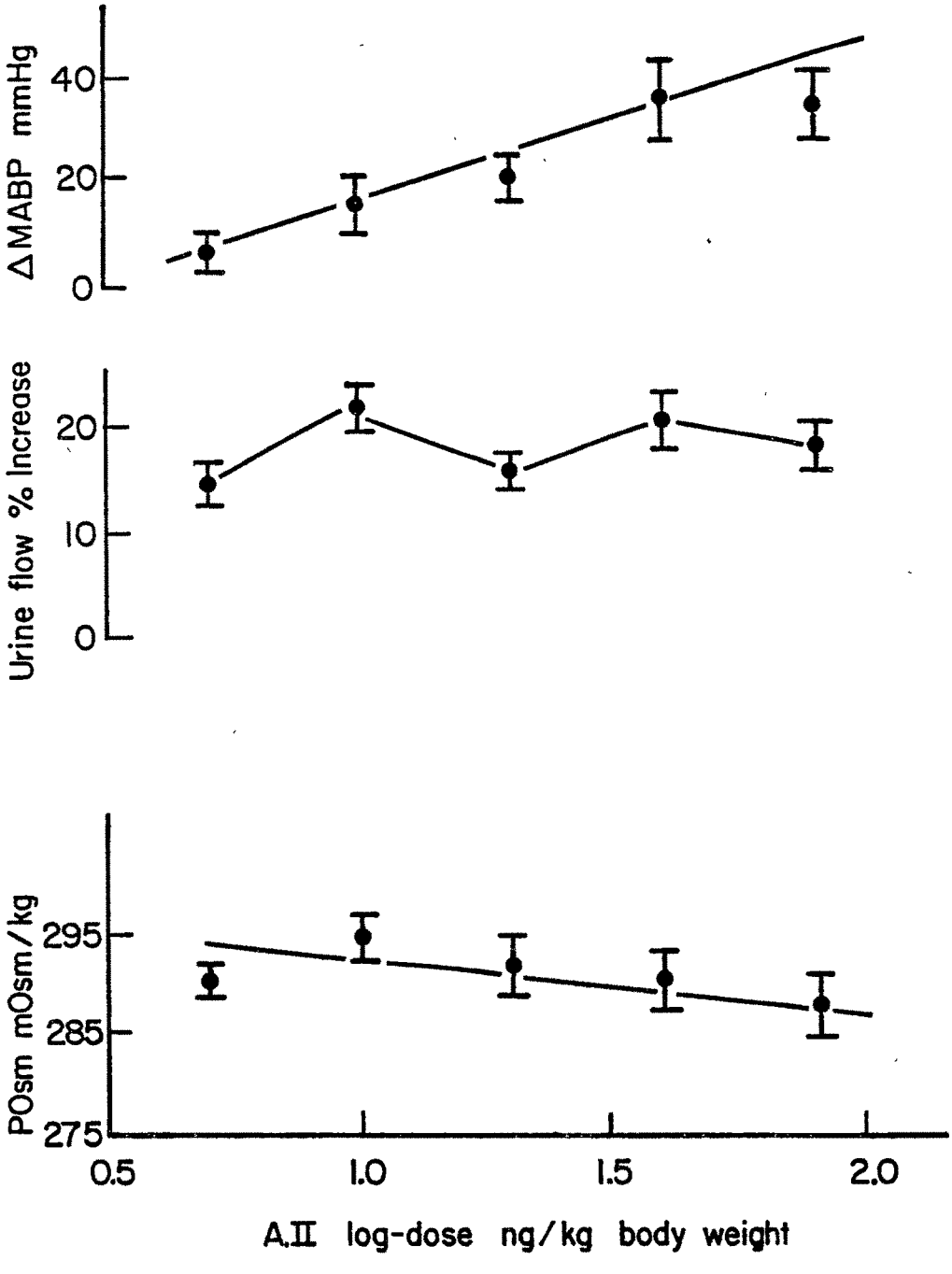

ΔMABP mmHg
40
20
0
Urine flow % Increase
20
10
0
POsm mOsm/kg
295
285
275
0.5
1.0
1.5
2.0
A.II log-dose ng/kg body weight

Figure 14

Dose-response curve of norepinephrine-induced ADH release in dogs. Each point is the mean of 4 observations ± S.E.M.

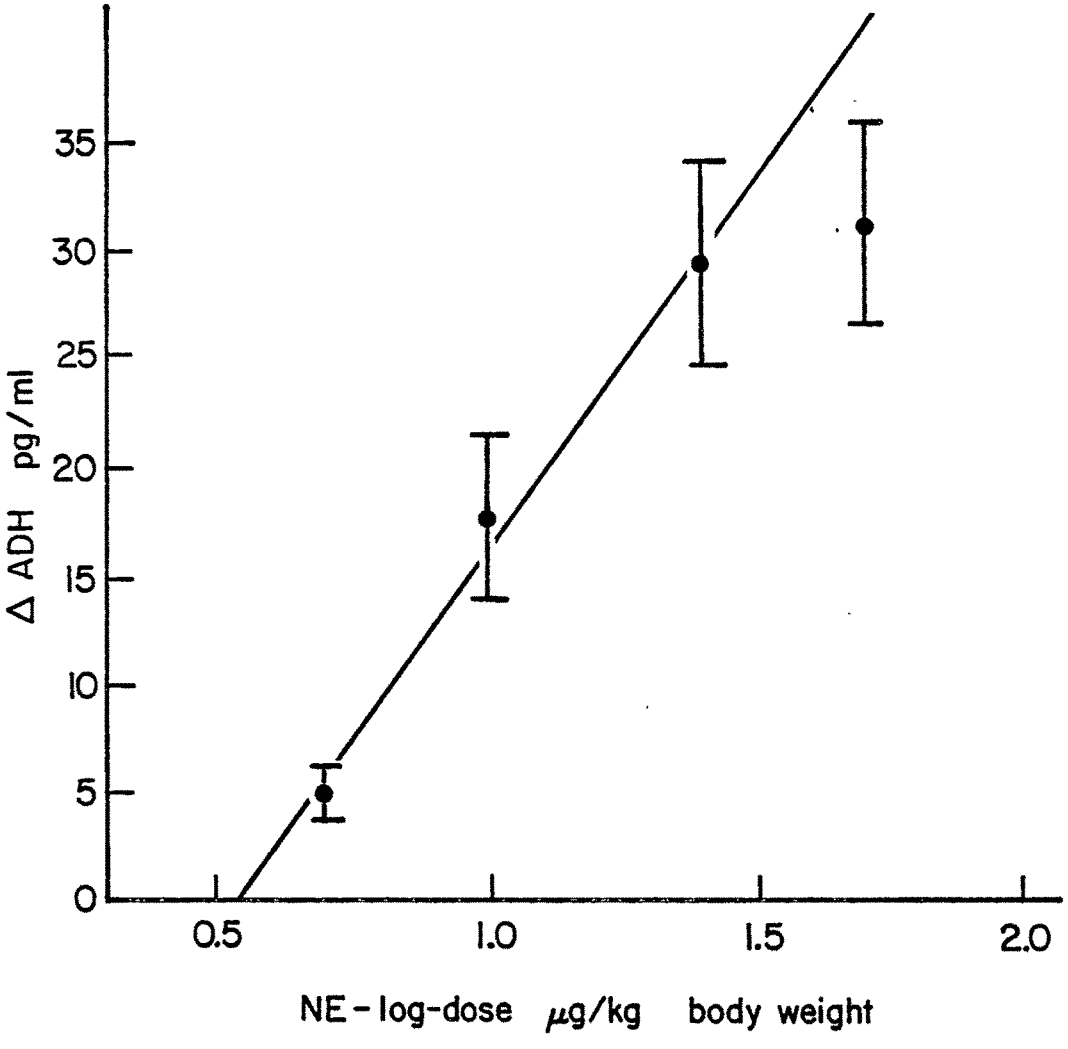
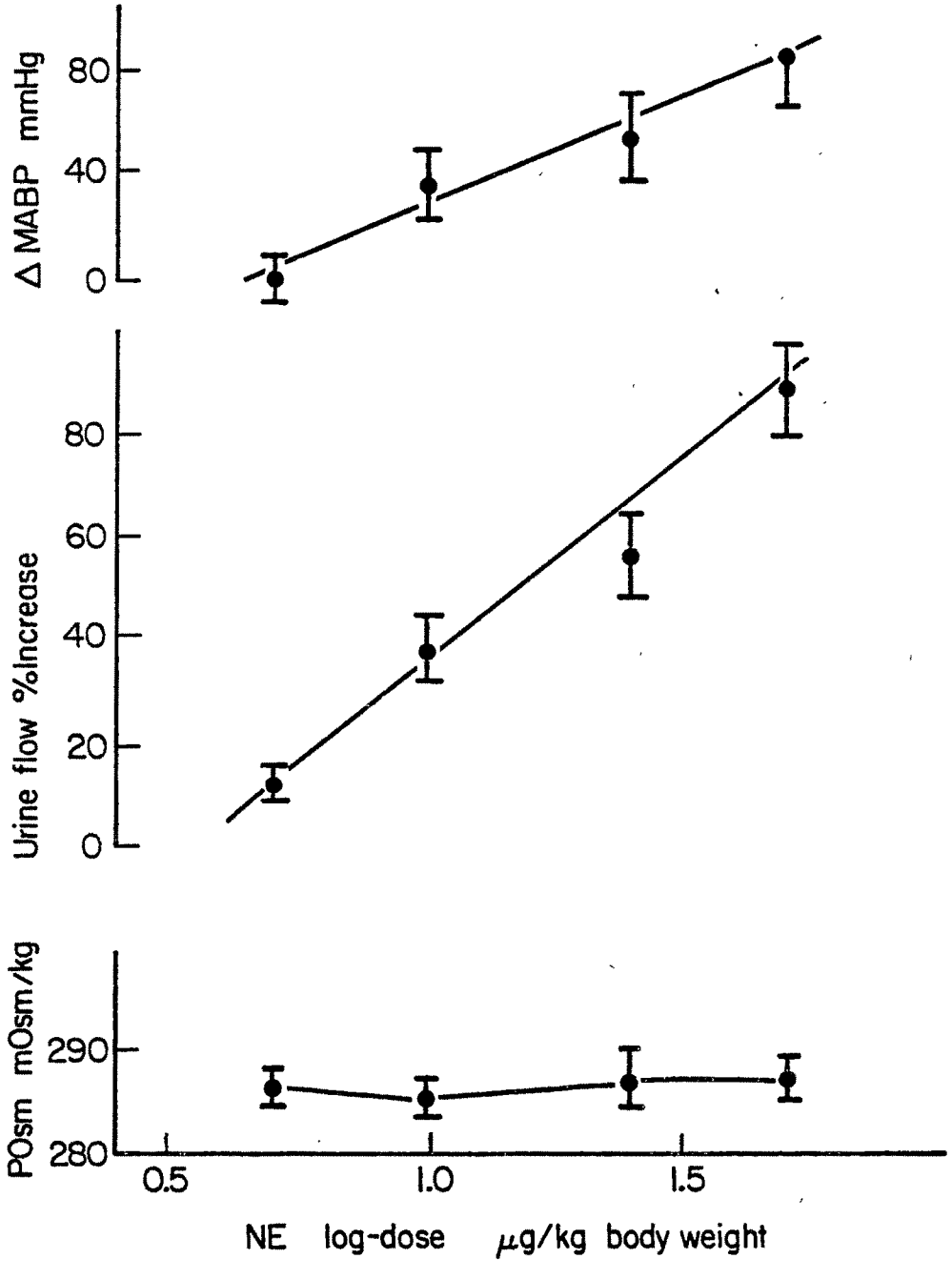

35
30
25
20
15
10
5
0
Δ ADH pg/ml
0.5
1.0
1.5
2.0
NE - log-dose μg/kg body weight

Figure 15

Changes in mean arterial blood pressure, urine flow and plasma osmolality of dog induced by norepinephrine. Each point is the mean of 4 observations ± S.E.M.


Δ MABP mmHg
80
40
0
Urine flow %Increase
80
60
40
20
0
POsm mOsm/kg
290
280
0.5
1.0
1.5
NE log-dose μg/kg body weight

The block of ADH release by mecamylamine was statistically significant ($p < 0.01$). However, the rise in plasma osmolality was unaffected by the drug; i.e., the increase was 10.6 ± 1.12 mOsm/kg and 10.8 ± 2.69 mOsm/kg respectively before and after the drug ($p > 0.05$) (Fig. 16).

2. Pentolinium

Pentolinium in a dose of 5 mg/kg body weight produced an effect similar to that seen with mecamylamine. Here the rise in plasma ADH level in response to hyperosmotic stimulation (3 M NaCl) was 27.8 ± 9.3 pg/ml prior to infusion of pentolinium. However, after the drug the same amount of NaCl could elicit a release only of 2.20 ± 0.71 pg/ml. This block of the ADH release by pentolinium was statistically significant ($p < 0.05$). Like mecamylamine, pentolinium did not alter the plasma osmolality (Δ P Osm, 21.25 ± 10.26 before and 15.50 ± 4.56 after the drug respectively, $p > 0.05$) (Fig. 17).

3. Phentolamine (Regitine)

Regitine, at a dose of 0.8 to 1.0 mg/kg body weight blunted the rise in plasma ADH level, induced by hyperosmotic stimulation. In the absence of the drug the infusion of hypertonic NaCl (3 M) elevated the plasma ADH by 7.0 ± 1.64 pg/ml. When the same osmotic stimulation was applied after the drug, the change in plasma ADH was only 0.4 ± 0.99 pg/ml ($p < 0.003$). Again the plasma osmolality rise induced by hyperosmotic saline was unchanged after regitine (Δosm 16.67 ± 2.91 before and 15.67 ± 3.38 after, $p < 0.05$) (Fig. 18).

FIGURE 16

Effect of mecamylamine on ADH release and plasma osmolality induced by hyperosmotic solution of 3 M NaCl. On the left is depicted the change in ADH level induced by 3 M NaCl before and after the drug while on the right is depicted the simultaneous rise in plasma osmolality induced by 3 M NaCl before and after the drug. Each point is the mean of 4 observations $\pm$ S.E.M.

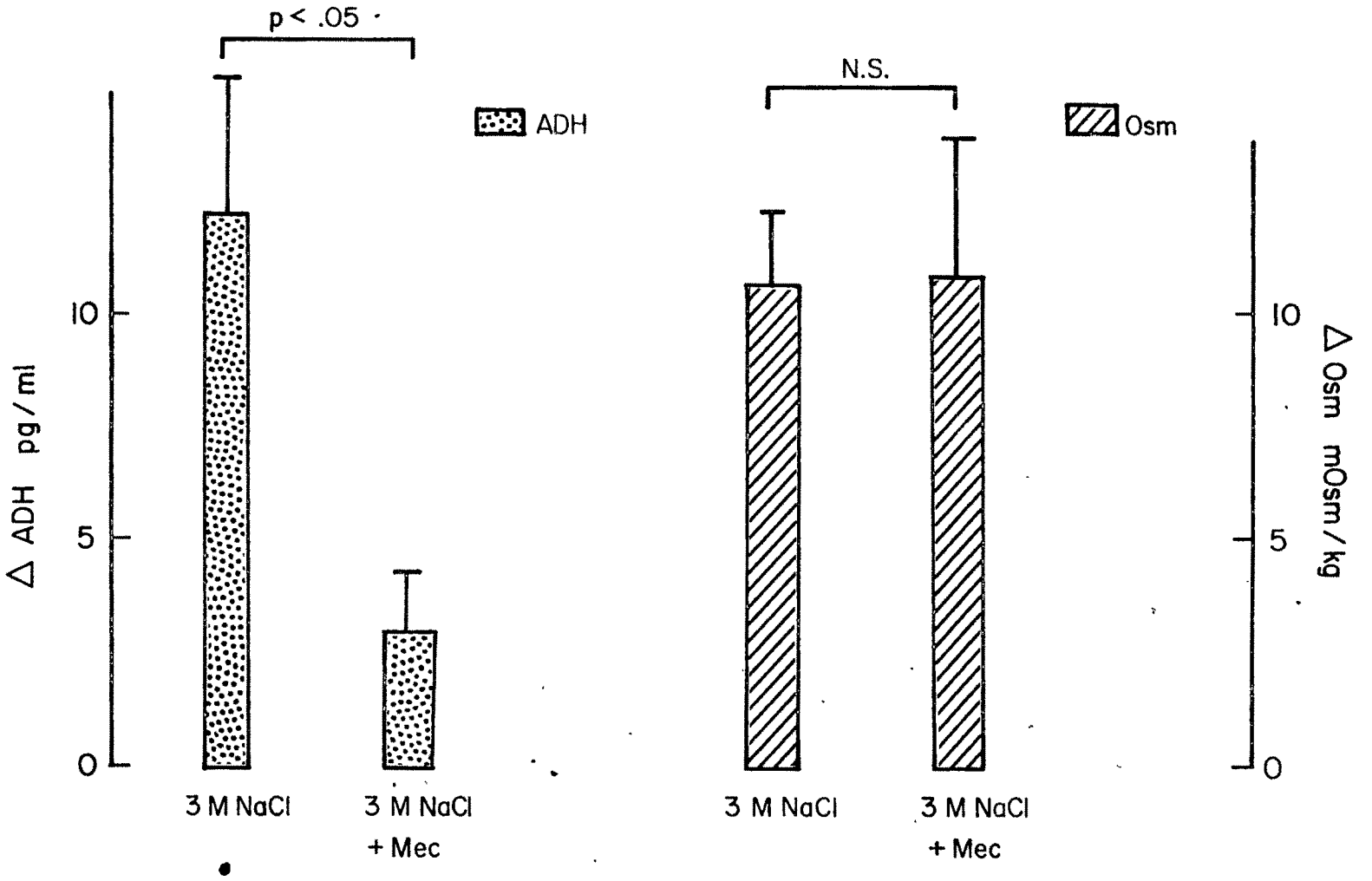

p < .05
N.S.
ADH
Osm
Δ ADH pg/ml
Δ Osm mOsm/kg
10
5
0
3 M NaCl
3 M NaCl + Mec
3 M NaCl
3 M NaCl + Mec

Figure 17

Effect of pentolinium on ADH release and plasma osmolality induced by hyperosmotic solution of 3 M NaCl. See Fig. 16 for explanation. Each point is the mean of 5 experiments ± S.E.M.

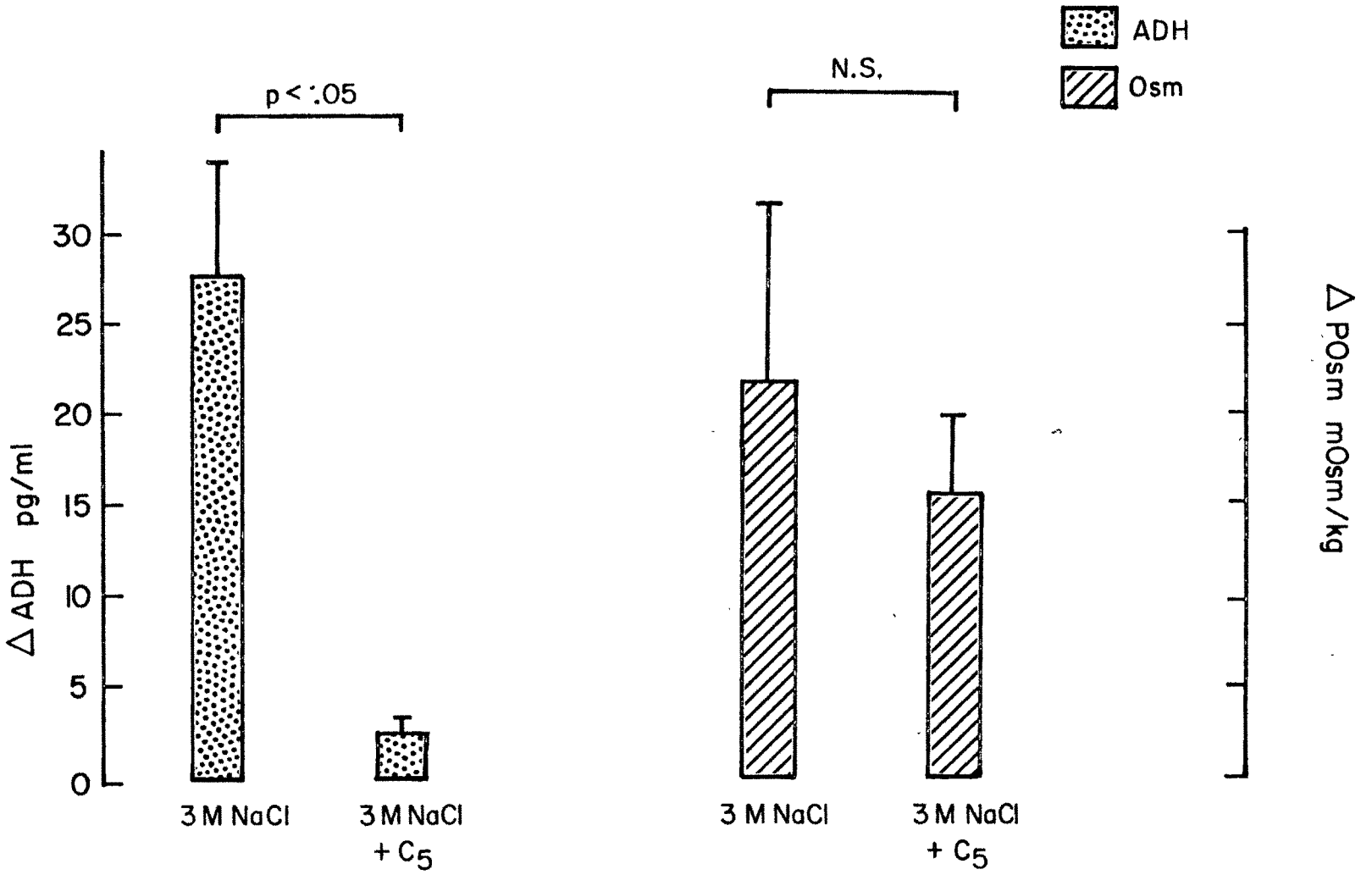
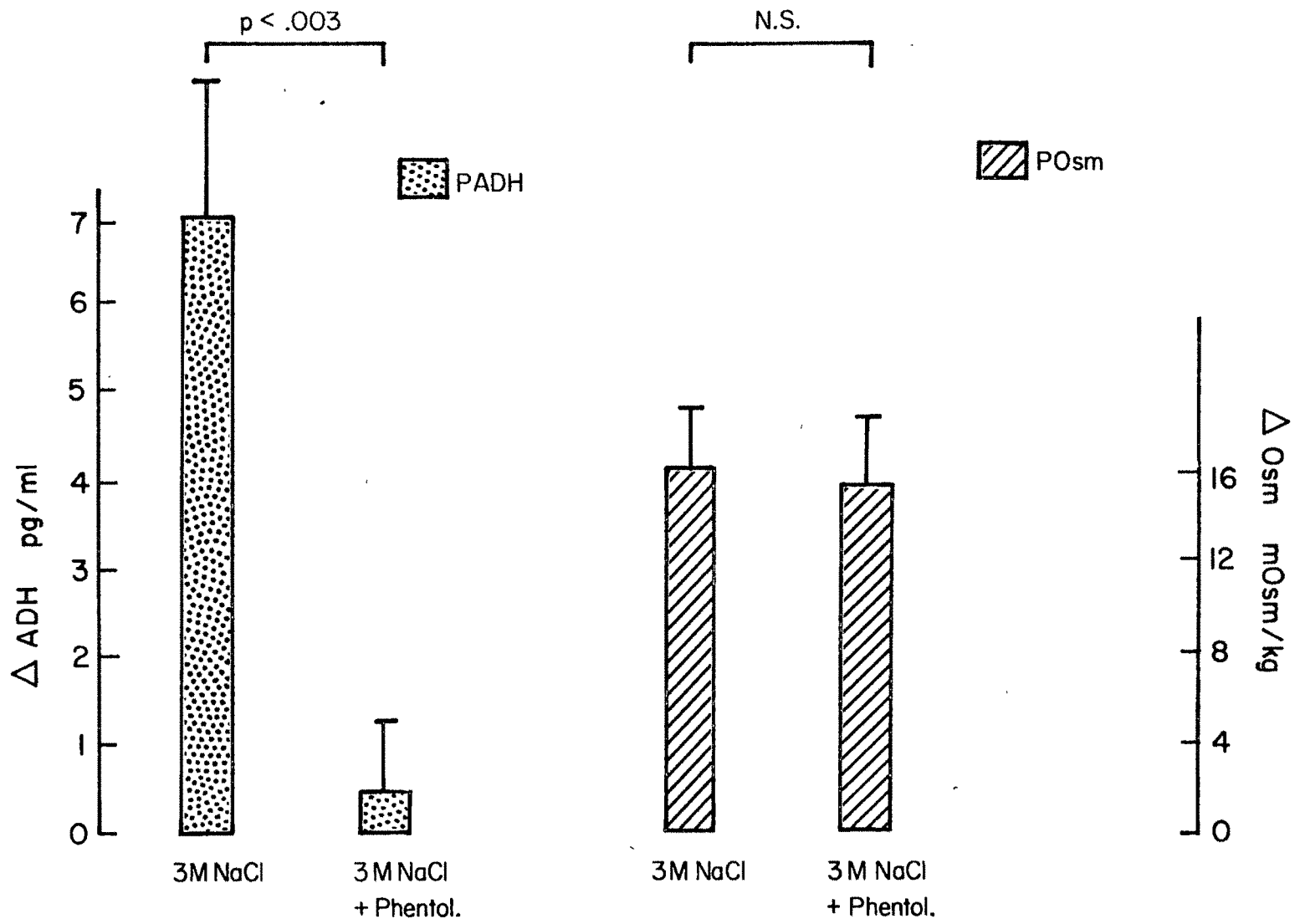

ADH
Osm
p < .05
N.S.
Δ ADH pg/ml
30
25
20
15
10
5
0
3 M NaCl
3 M NaCl + C5
3 M NaCl
3 M NaCl + C5
Δ POsm mOsm/kg

Figure 18

Effect of phentolamine on ADH release and plasma osmolality induced by hyperosmotic solution of 3 M NaCl. See Fig. 16 for explanation. Each point is the mean of 5 experiments ± S.E.M.

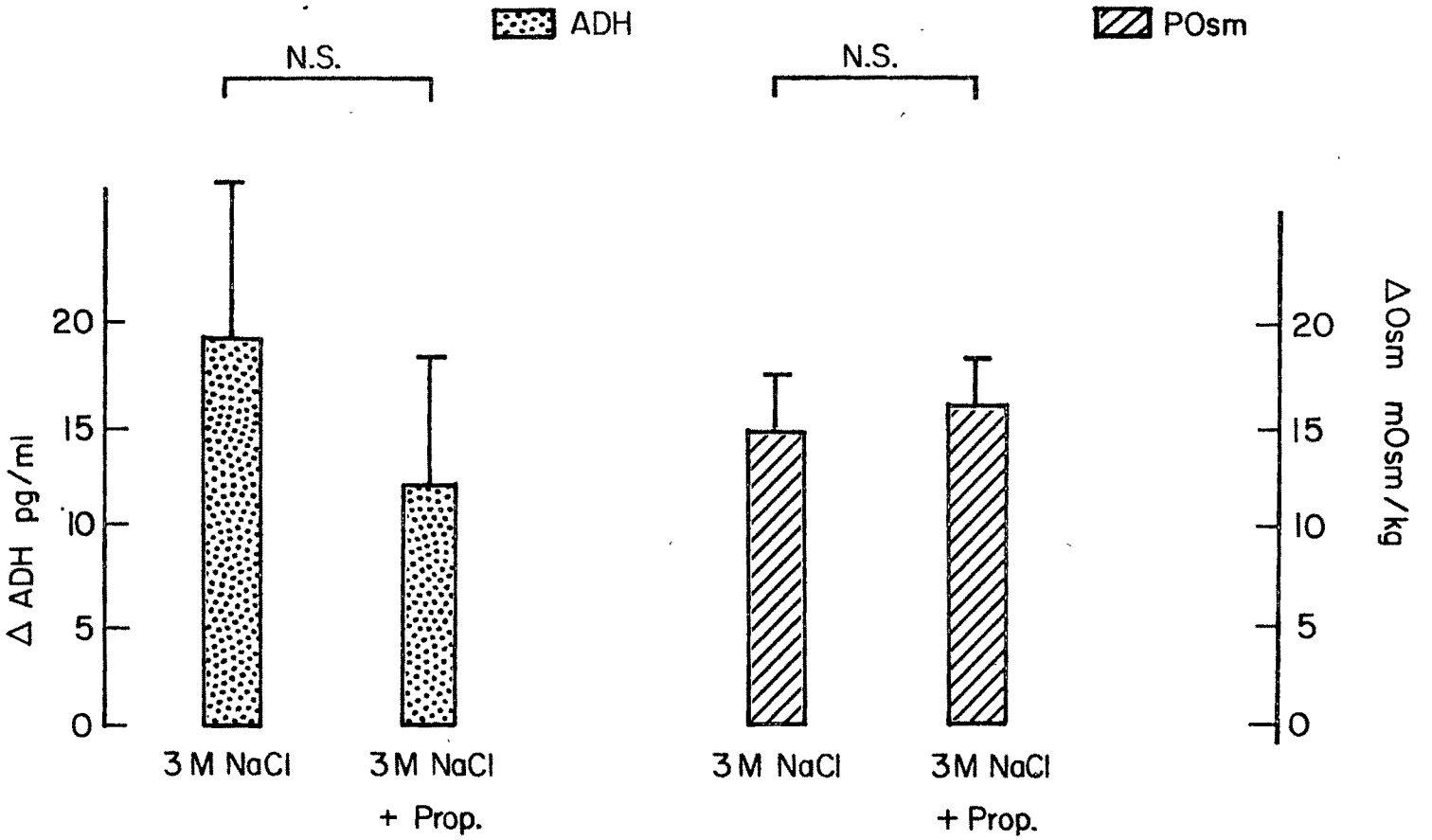

p < .003
N.S.
PADH
POsm
Δ ADH pg/ml
0
1
2
3
4
5
6
7
Δ Osm mOsm/kg
0
4
8
12
16
3M NaCl
3 M NaCl
+ Phentol.
3M NaCl
3 M NaCl
+ Phentol.

4. Propranolol

Propranolol, at a dose of 0.6 to 1.0 mg/kg body weight did not affect the release of ADH induced by hypertonic saline. The plasma ADH level increased by 19.9 $\pm$ 8.15 pg/ml after hyperosmotic stimulation (3 M NaCl) alone. After hyperosomotic stimulation (3 M NaCl) propranolol and the change in ADH level was 12.36 $\pm$ 5.74 pg/ml ($p > 0.05$). The rise in plasma osmolality was similar before and after propranolol (14.4 $\pm$ 2.8, 16.0 $\pm$ 2.07; $p > 0.05$) (Fig. 19).

Figure 19

Effect of propranolol on ADH release and plasma osmolality induced by hyperosmotic solution of 3 M NaCl. See Fig. 16 for explanation. Each point is the mean of 5 experiments ± S.E.M.


ADH
POsm
N.S.
N.S.
Δ ADH pg/ml
0
5
10
15
20
ΔOsm mOsm/kg
0
5
10
15
20
3 M NaCl
3M NaCl
+ Prop.
3 M NaCl
3M NaCl
+ Prop.

Results - Part II

In vitro experiments

A. Oxygen uptake by isolated SONH

The ability of the SONH to consume oxygen from the chamber was constant up to a period of 150 min after which it slowly declined by 10 and 20%. All of these preparations studied behaved in an identical manner.

B. Control study

Fig. 20 shows the basal release of ADH by SONH exposed to a medium having an osmolality of 300 mOsm/kg. When the change in ADH concentration across time was analyzed, there was no significant increase ($p > 0.05$).

C. Stimulants

1. Acetylcholine (Ach)

Ach, at a concentration of 1.38×10^{-5} M caused an increase in ADH release from 17.33 ± 5.40 pg/min to 41.5 ± 10.8 pg/min, and then returned to basal level of 15.9 ± 3.29 pg/min when the drug was removed from the incubation medium. This increase in release of ADH, when compared to the control value was statistically significant ($p < 0.002$) (Fig. 21). A higher concentration of Ach, 6.9×10^{-5} M also increased the release of ADH from 16.4 ± 10.5 pg/min to 27.7 ± 13.7 pg/min; but it was not significant statistically ($p > 0.05$).

Figure 20

Basal release of ADH by SONH during the experimental phase, as de-described in the methods. The preparation is incubated in an isotonic medium of an osmolality of 300 mOsm kg., and the ADH released into the medium during the 3 fifteen minute periods is measured. This group will serve as the control for the subsequent study using drugs. Period 1, represens the base line, period 2, the experimental where appropriate drugs are added and period 3, the recovery from the effect of the drug. Each period represents the mean of 5 observations $\pm$ S.E.M.

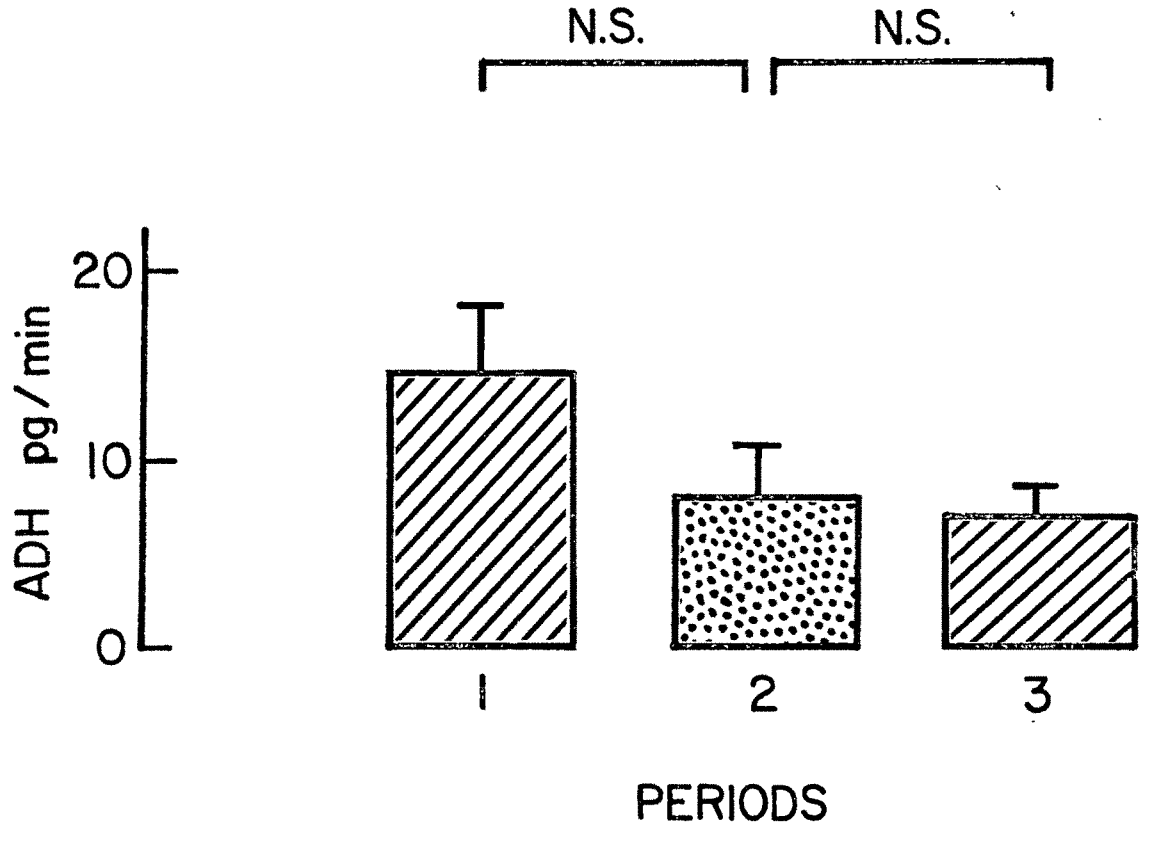
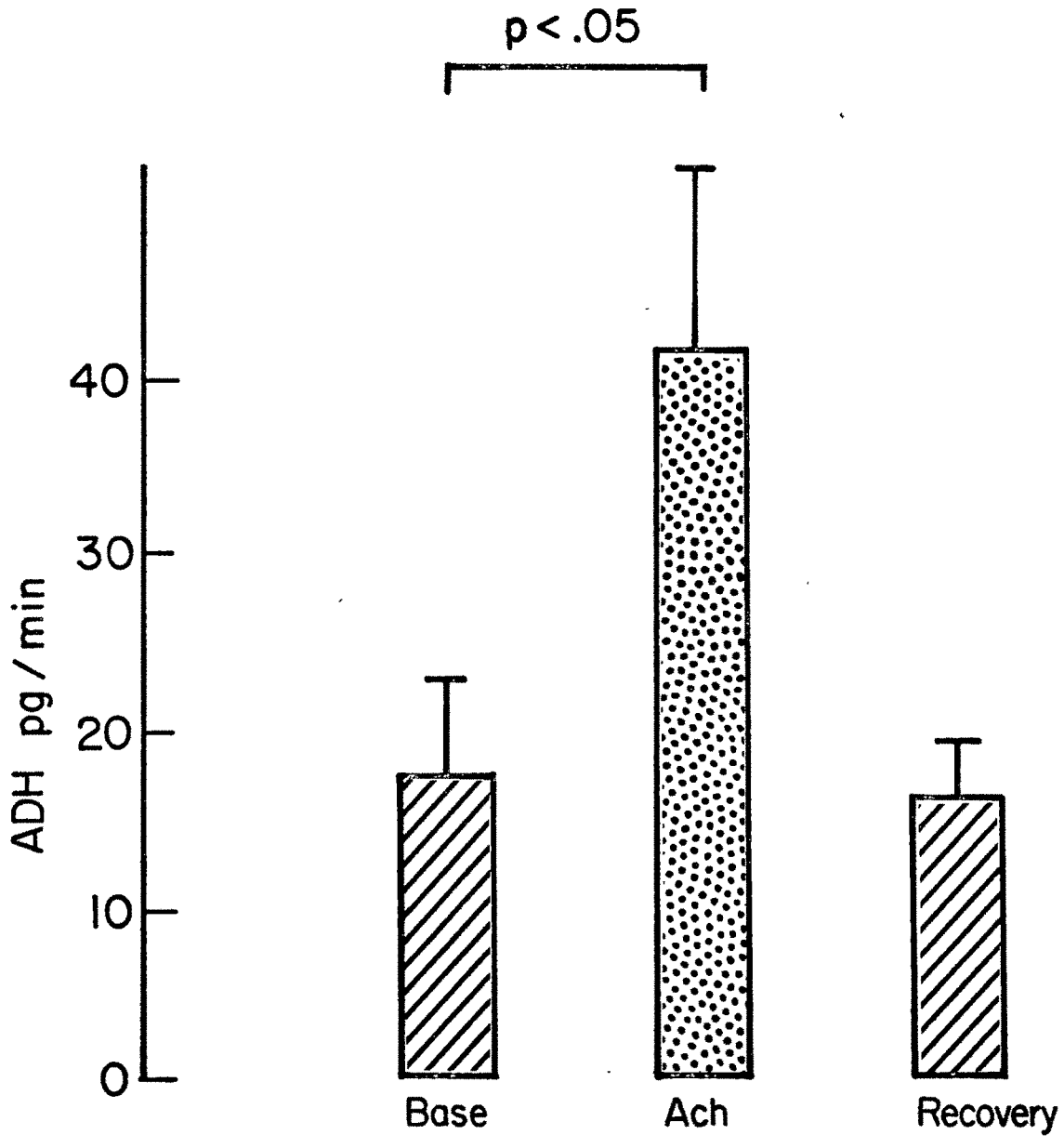

N.S.
N.S.
ADH pg/min
20
10
0
1
2
3
PERIODS

Figure 21

Ach-induced release of ADH from SONH. The first period represents the basal release of ADH by the preparation. During the second period, Ach was added in the incubation medium and during the third period, the preparation was rinsed off the drug. This period represents the recovery of the preparation. Each point represents the mean of 5 observations $\pm$ S.E.M.


p < .05
ADH pg / min
40
30
20
10
0
Base
Ach
Recovery

2. Nicotine

Nicotine, at a concentration of 3.08×10^{-5} M elicited an increase in ADH release like Ach. With nicotine present the ADH concentration was 18.8 ± 4.1 pg/min. After removal of nicotine the ADH level fell to 9.74 ± 2.94 pg/min (Fig. 22). The increase in ADH release produced by nicotine was statistically significant when compared to the control value ($p < 0.05$)

3. McN-A-343-11

In contrast to Ach and nicotine, McN-A-343-11, at a concentration of 3.15×10^{-5} M did not increase the release of ADH (ADH released with McN-A-343-11 was 19.4 ± 6.2 pg/min; control ADH, i.e. without McN-A-343-11 was 12.8 ± 3.5 pg/min; $p > 0.05$) (Fig. 23).

4. Angiotensin-11 (A-11)

A-11 at a concentration of 9.7×10^{-7} M increased the release of ADH from 12.66 ± 4.39 to 20.46 ± 5.06 pg/min. This increase was statistically significant ($p < 0.01$) (Fig. 24).

5. Isoproterenol (IP)

IP, at a concentration of 2.37×10^{-5} M had no effect. But, at higher concentration (11.85×10^{-5} M), ADH release increased. However, this change was not significant when compared to the control release ($p > 0.05$) (Fig. 25).

6. Noradrenaline (NA)

In contrast to IP, NA at 1.48×10^{-5} M and 7.38×10^{-5} M, increased ADH release and this increase was significant ($p < 0.001$ at

Figure 22

Nicotine-induced release of ADH from SONH. The experimental protocol is the same as described in Fig. 21. Each point is the mean of 5 observations ± S.E.M.

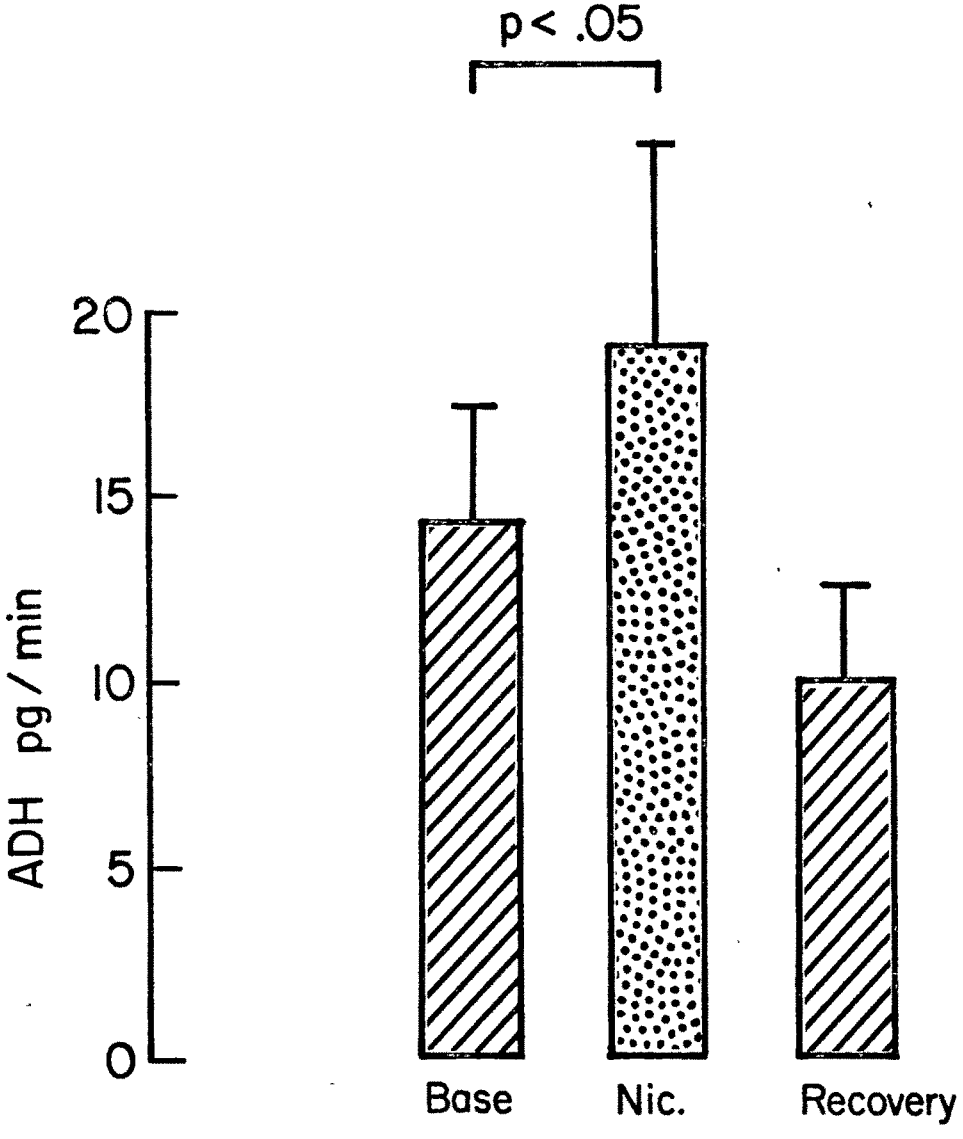

p< .05
20
15
10
5
0
ADH pg / min
Base
Nic.
Recovery

Figure 23

McN-A-343-11-induced release of ADH from SONH. The experimental protocol is the same as described in Fig. 21. Each point is the mean of 4 observations ± S.E.M.

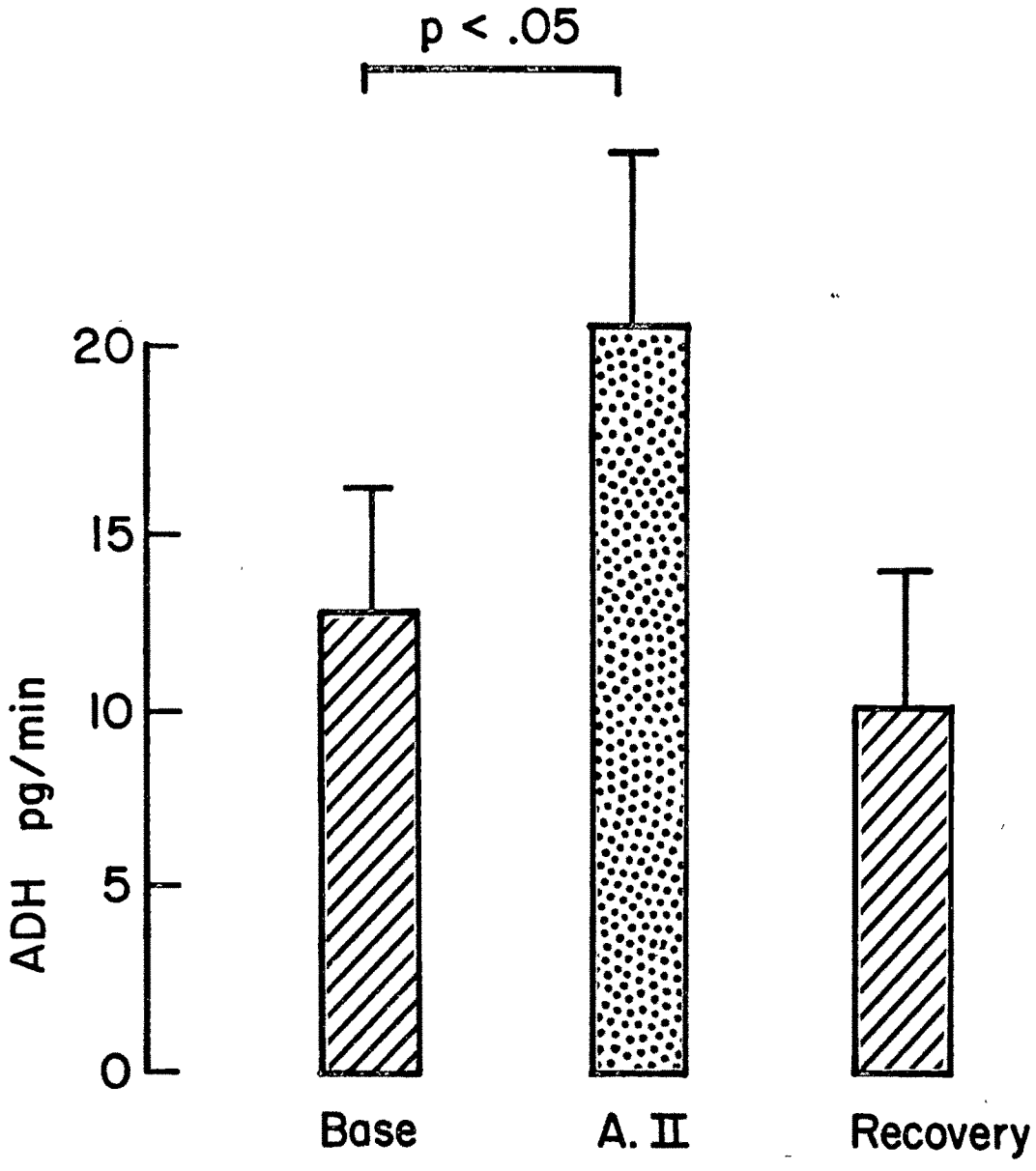

p < .05
20
15
10
5
0
ADH pg/min
Base
A. II
Recovery

Figure 24

A-II-induced release of ADH from SONH. The experimental protocol is the same as described in Fig. 21. Each point is the mean of 4 observations ± S.E.M.

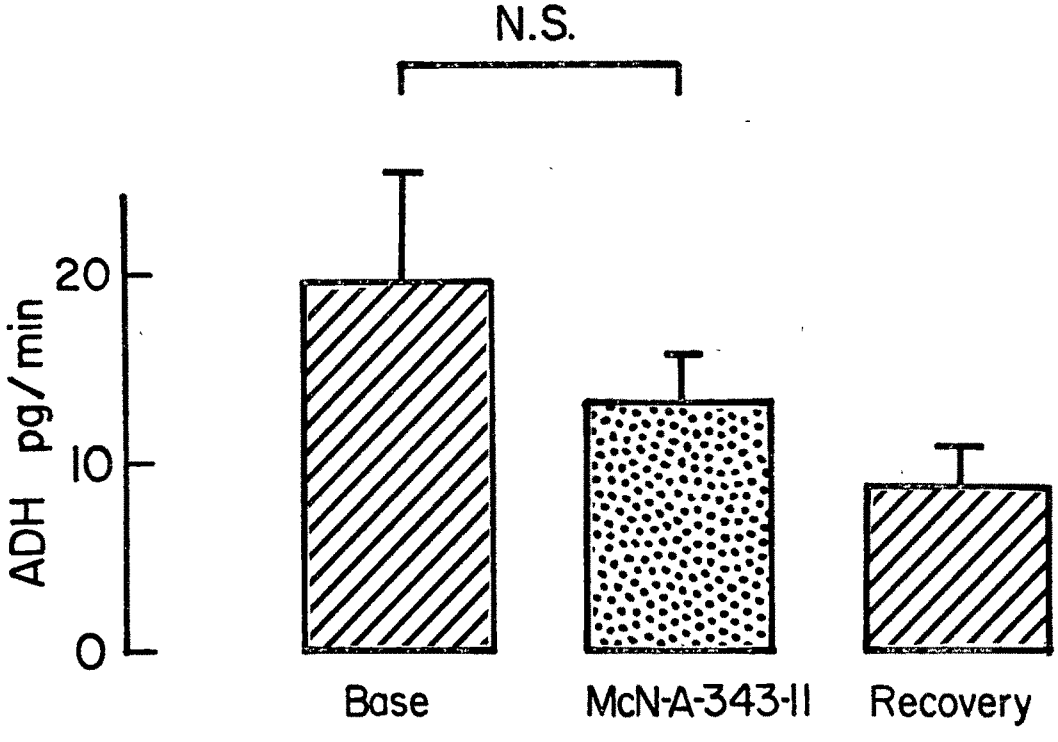

N.S.
ADH pg/min
20
10
0
Base
McN-A-343-11
Recovery

Figure 25

Isoprenaline-induced release of ADH from SONH. The experimental protocol is the same as described in Fig. 21. Each point is the mean of 4 observations ± S.E.M.

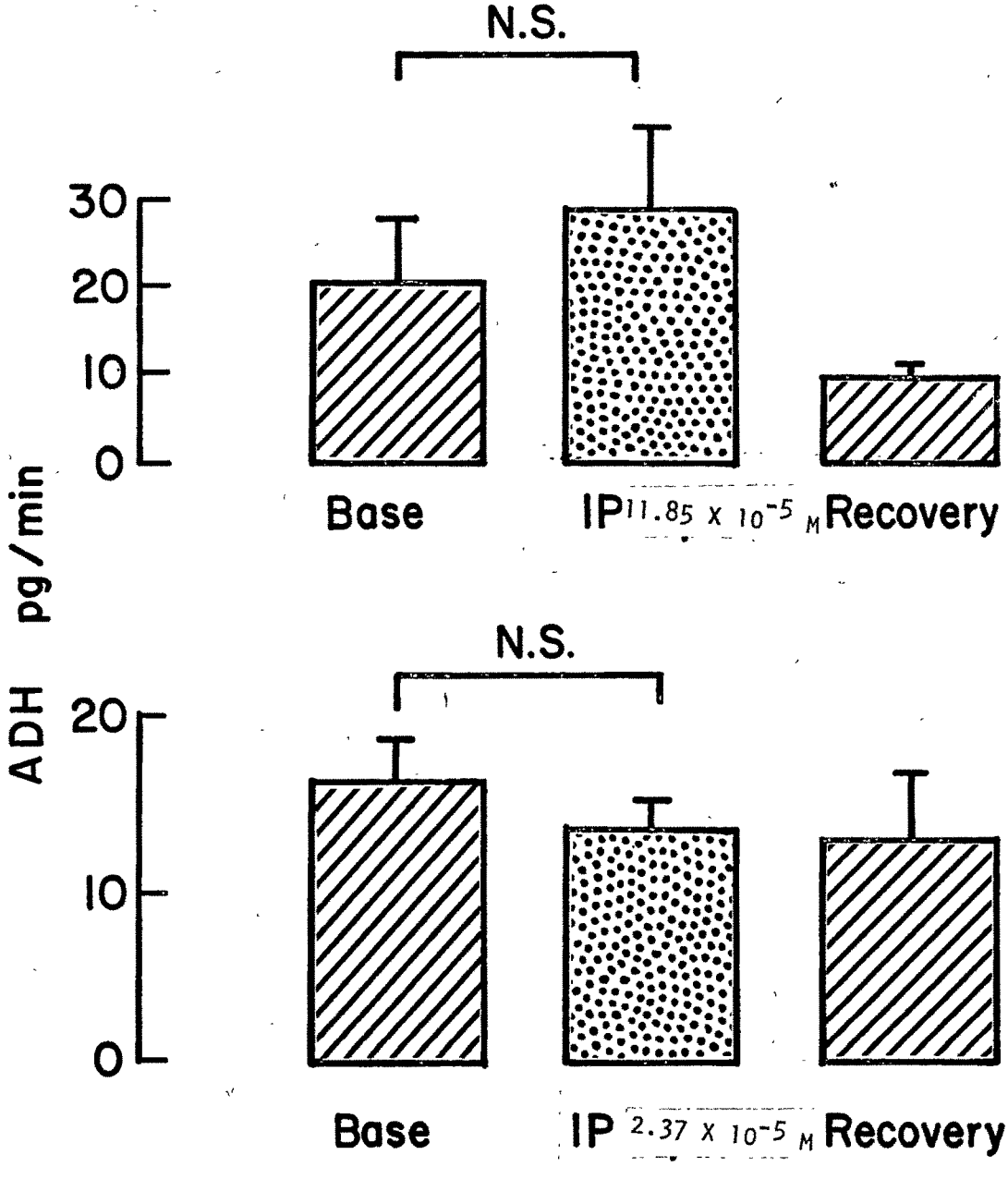
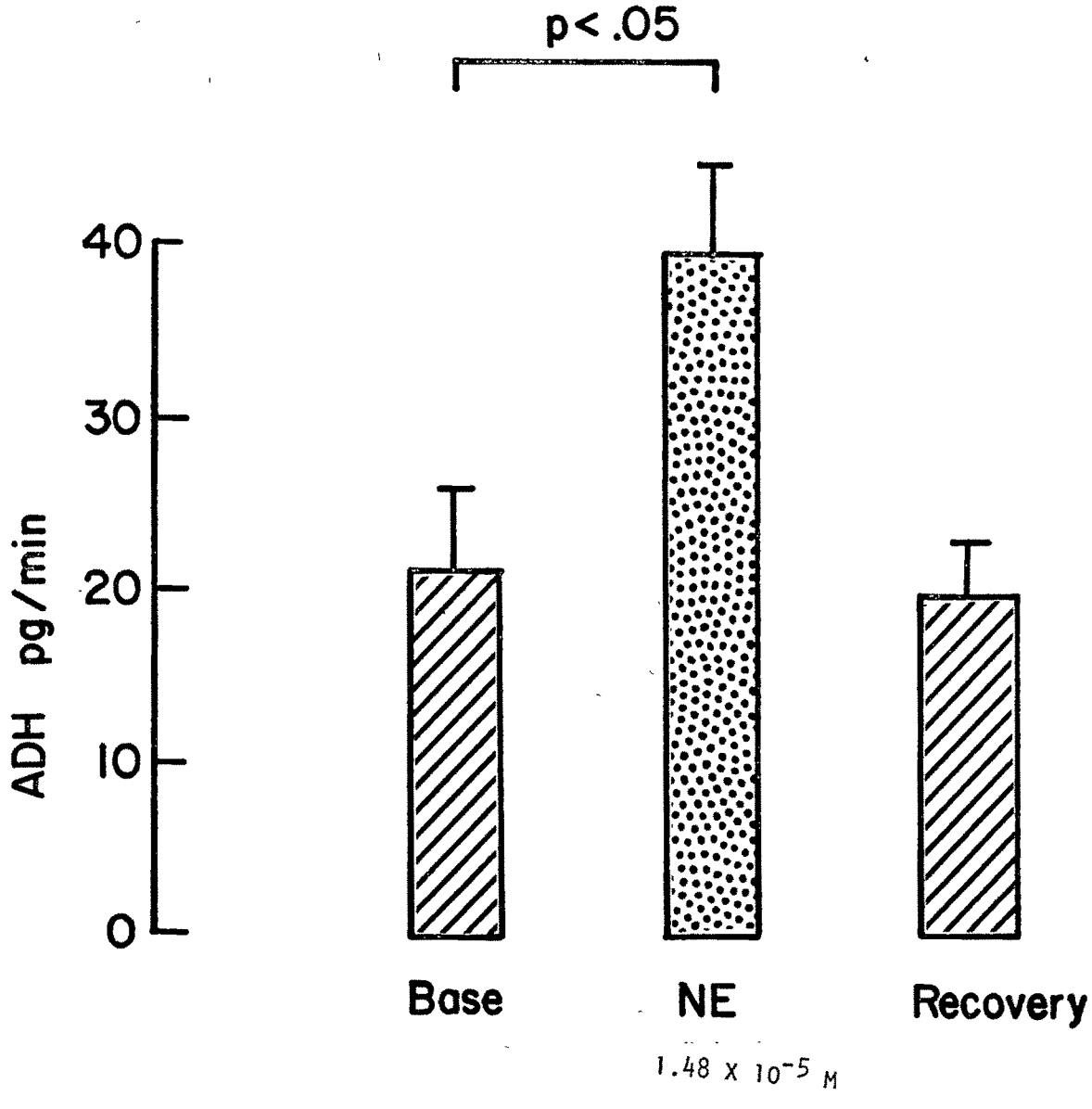
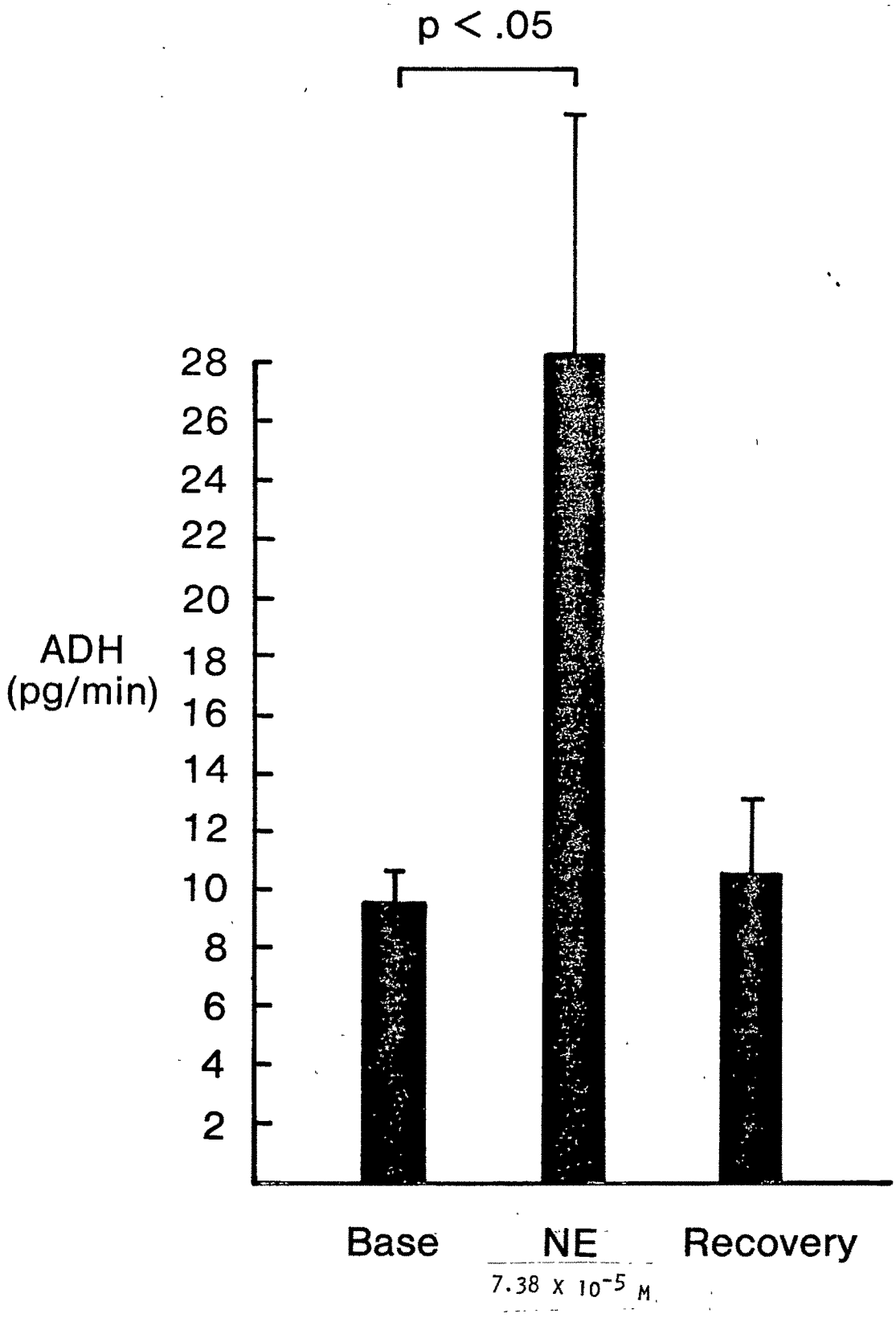

N.S.
30
20
10
0
ADH pg/min
Base
IP 1.85 X 10-5 M
Recovery
N.S.
20
10
0
Base
IP 2.37 X 10-5 M
Recovery

Figure 26

Norepinephrine-induced release of ADH from SONH. The experimental protocol is the same as described in Fig. 21. Each point is the mean of 4 observations ± S.E.M.


p< .05
40
30
20
10
0
ADH pg/min
Base
NE
1.48 X 10-5 M
Recovery

STIMULATION OF ADH RELEASE BY NOREPINEPHRINE (NE) IN SONH



the lower doses and $p < 0.004$ at the higher doses) (Fig. 26).

D. Effect of osmotic stimulation on the release of ADH.

Fig. 27 shows the change in ADH release when the preparation was exposed to hyperosmotic solutions of 400, 500, 600, and 760 mOsm/kg NaCl. A significant correlation between bath osmolality and ADH release was found ($r = 0.614$; $p < 0.05$).

When profile analysis was used to compare ADH release during the exposure of SONH to a 300 mOsm bath with ADH release during exposure to a 400 mOsm bath, the difference in ADH release was found to be significant at the 6% level ($0.05 < p < 0.06$) (Fig. 28).

E. Effects of drugs on osmotic stimulation.

1. Mecamylamine

Fig. 29 shows the effect of ganglion blocker, mecamylamine on osmotic stimulation. When the preparation was exposed to mecamylamine at a concentration of 1.49×10^{-3} M, the drug blunted the release of ADH induced by 400 mOsm NaCl. When the changes in ADH release across time was analysed by profile analysis, there was no significant difference ($p > 0.05$). A lower concentration of the drug was without any effect on hyperosmotic stimulation.

2. Pentolinium

Pentolinium, like mecamylamine, at a concentration of 4.60×10^{-4} M was also effective in blocking the release of ADH induced by 400 mOsm NaCl (Fig. 30).

Figure 27

Correlation between osmolality and ADH release. The SONH was incubated in buffer of different osmolalities and the increase in ADH release were plotted against the corresponding osmolality. Regression analysis was performed and correlation coefficient (r) between the ADH release and osmolality of the medium is also expressed here. Each point represents the mean of 5 experiments $\pm$ S.E.M.

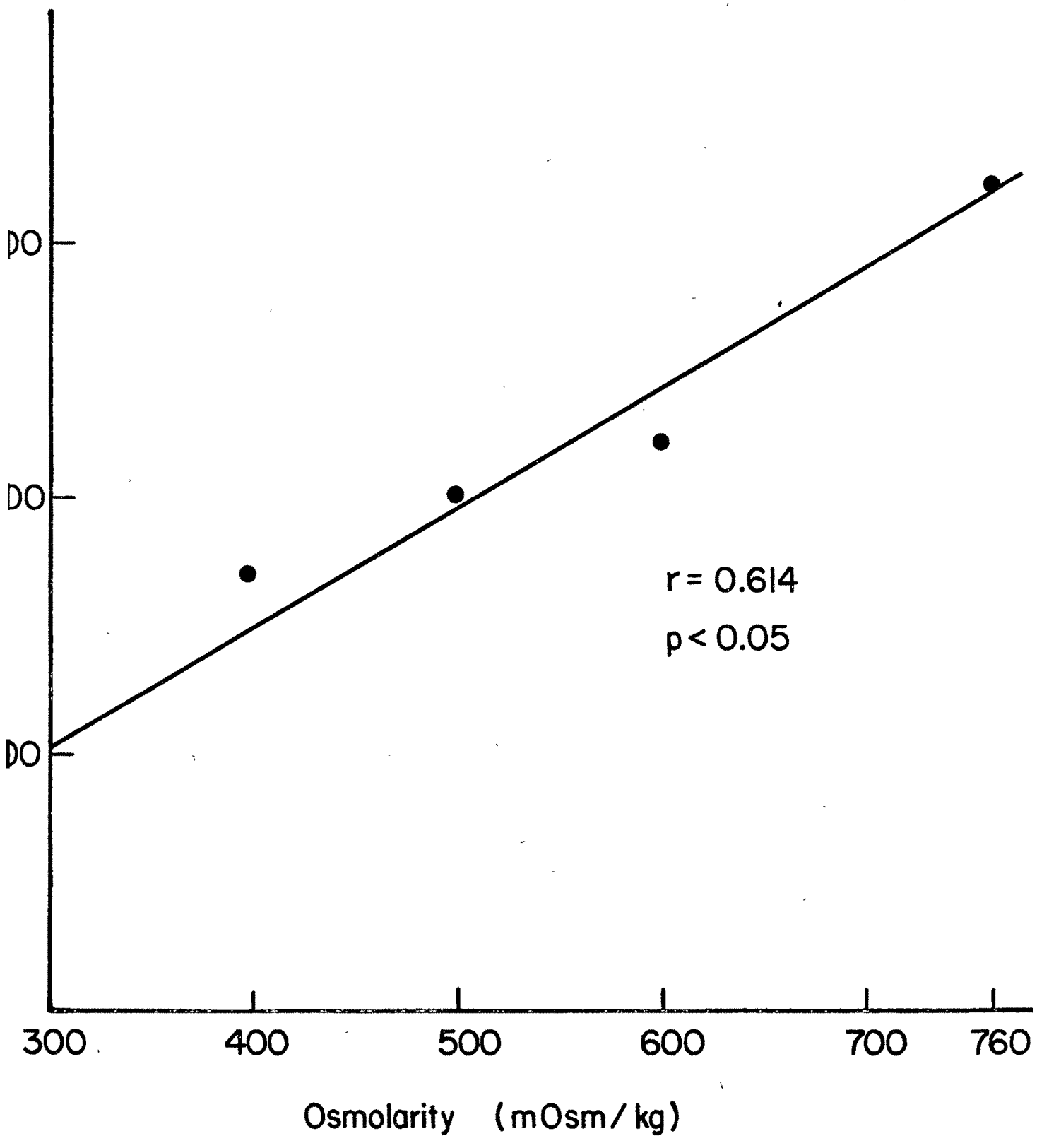

DO
DO
DO
r = 0.614
p < 0.05
300
400
500
600
700
760
Osmolarity (mOsm/kg)

Figure 28

ADH release from SONH induced by hypertonic NaCl. The basal release of ADH when the preparation is in the isotonic medium is depicted in the first period. During the second period, the media is replaced with hypertonic medium with an osmolality of 400 mOsm/kg. The third and fourth period represent recovery periods during which time the preparation is rinsed and incubated in isotonic medium. Each point is the mean of 5 observations $\pm$ S.E.M.

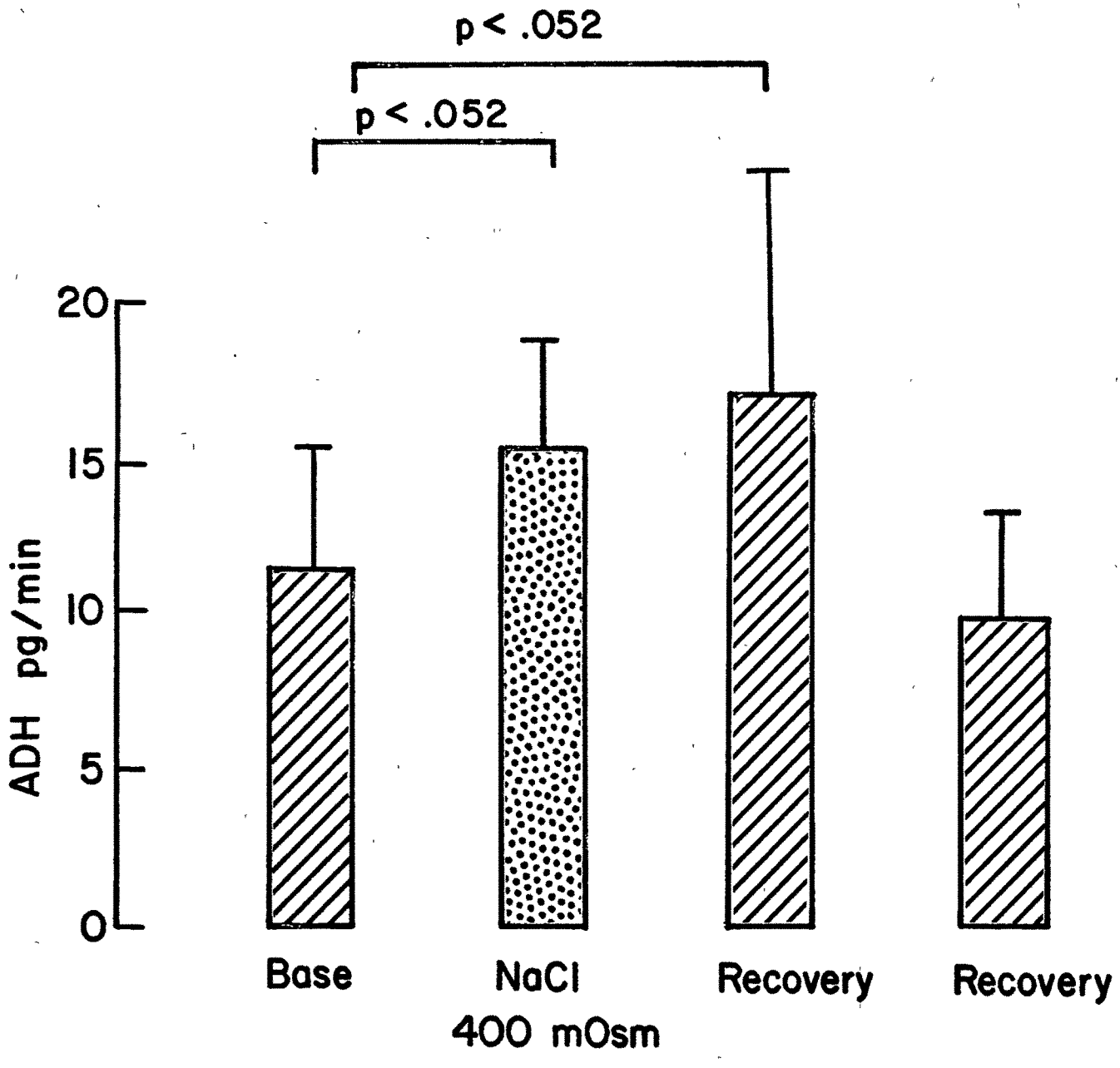

p < .052
p < .052
20
15
10
5
0
ADH pg/min
Base
NaCl
Recovery
Recovery
400 mOsm

Figure 29

Effect of mecamylamine on ADH release induced by hypertonic NaCl. Mecamylamine was added in the first period. During the second period the tissue is in the hypertonic medium containing 1.49×10^{-3} mecamylamine. At the end of the second period the preparation is rinsed with isotonic medium and allowed to recover. The last 2 periods represent the recovery periods. Each point is the mean of 5 observations $\pm$ S.E.M.

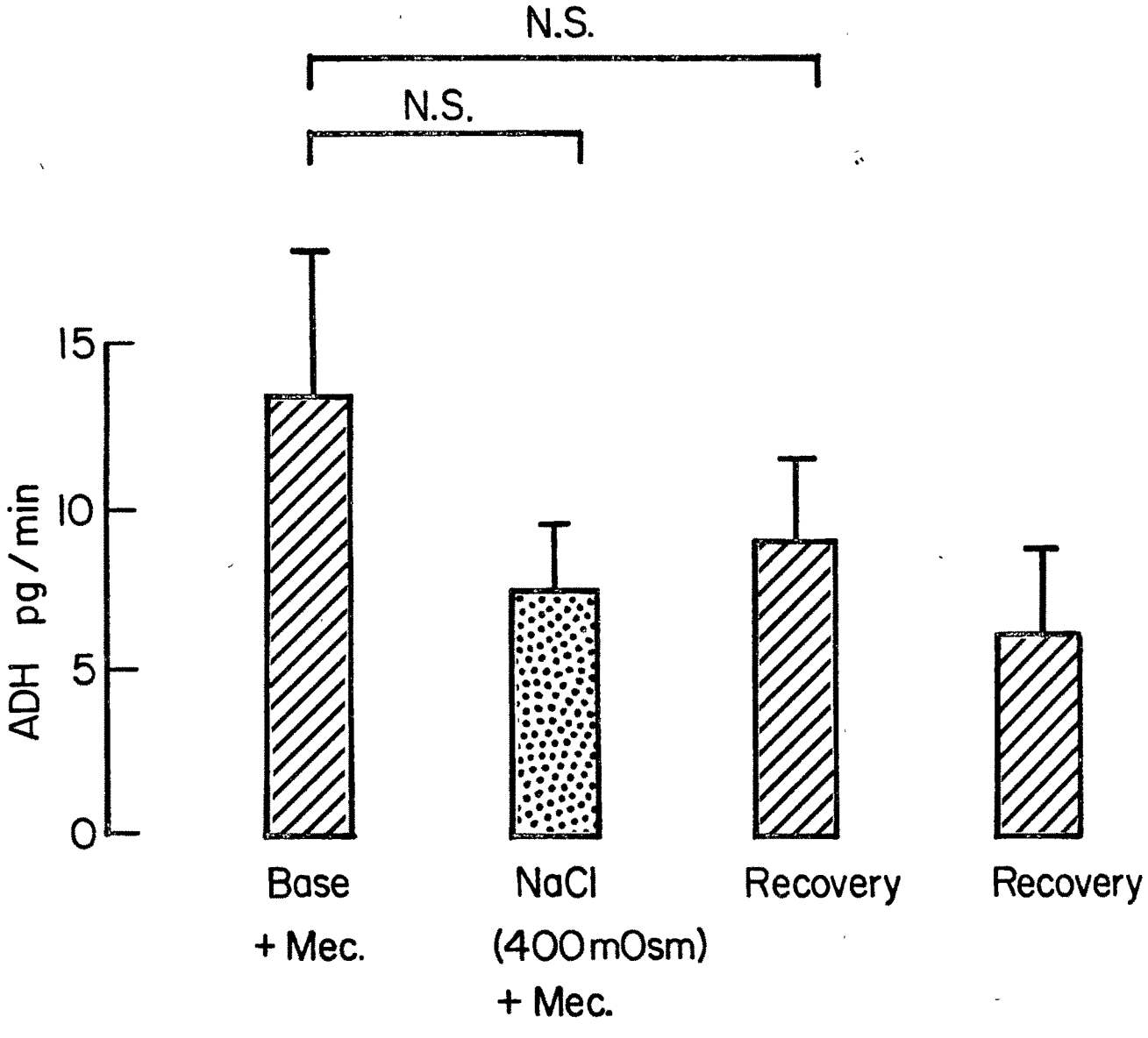
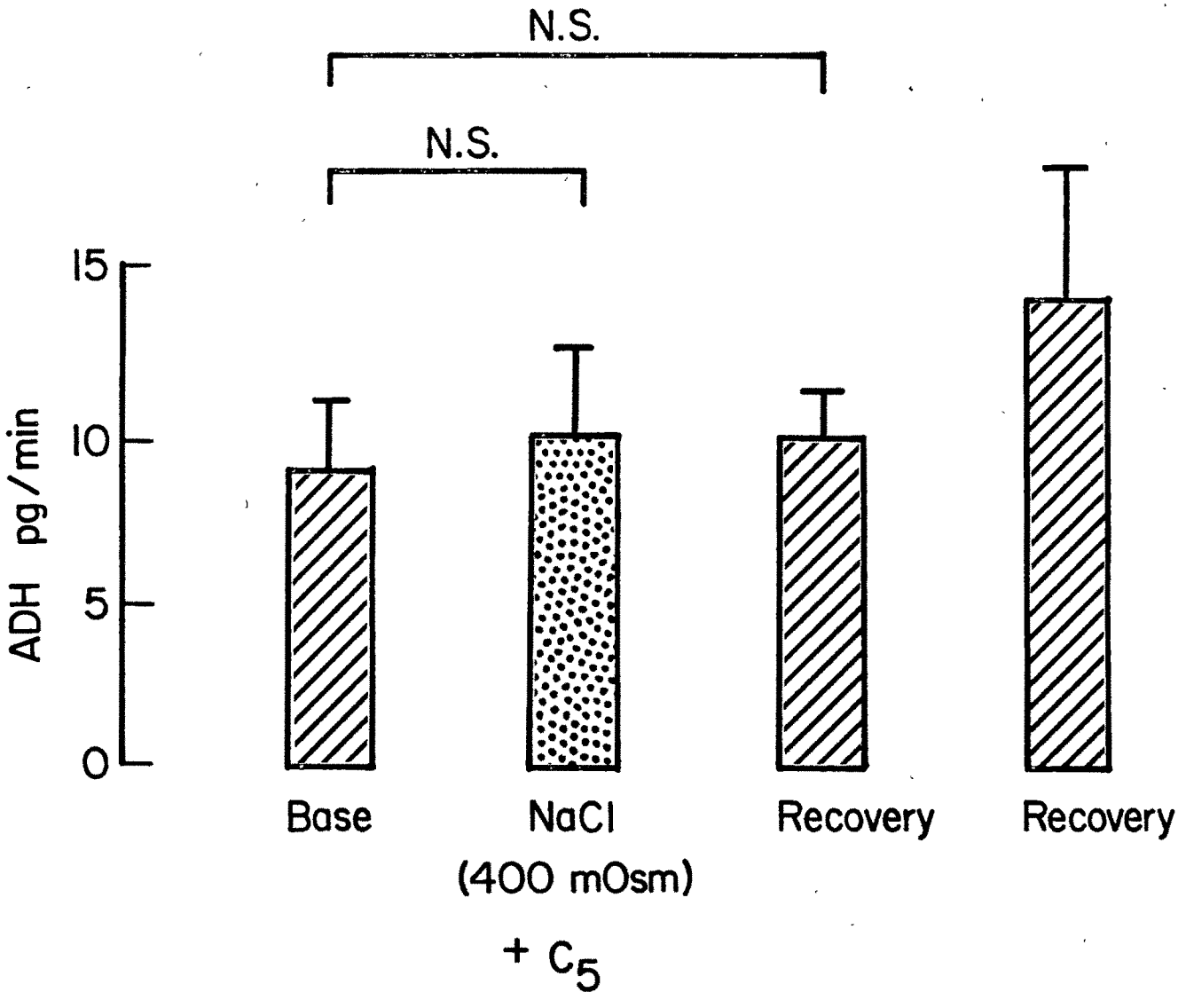

N.S.
N.S.
15
10
5
0
ADH pg/min
Base
+ Mec.
NaCl
(400mOsm)
+ Mec.
Recovery
Recovery

Figure 30

Effect of pentolinium on ADH release induced by hypertonic NaCl. The experimental protocol is the same as described in Fig. 29. Each point is the mean of 5 observations ± S.E.M.

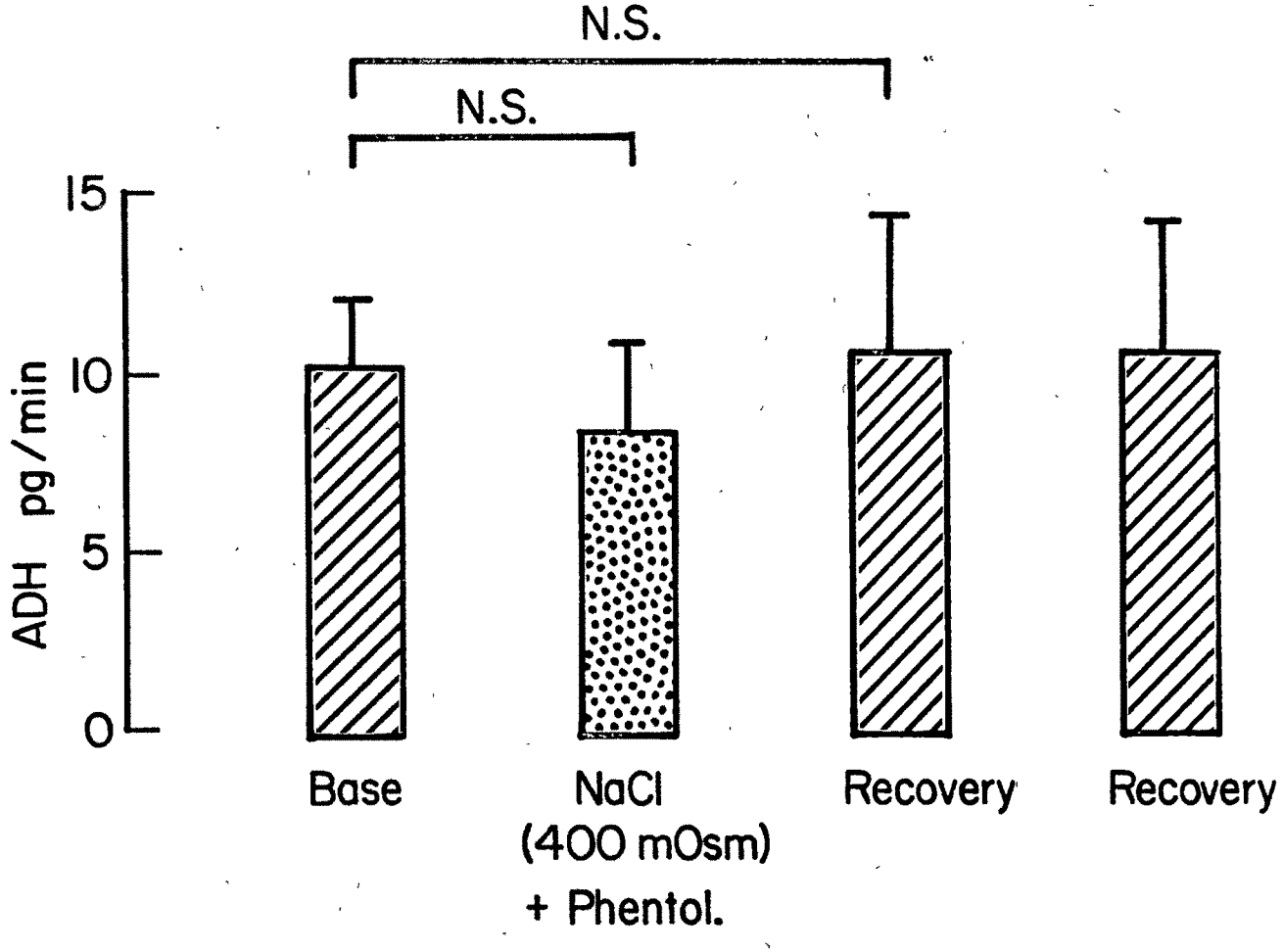

N.S.
N.S.
15
10
5
0
ADH pg/min
Base
NaCl
(400 mOsm)
+ C_5
Recovery
Recovery

3. Phentolamine

The α-blocking drug, phentolamine, at a concentration of 4.6×10^{-4} M also blocked the release of ADH induced by osmotic stimulation (Fig. 31).

Figure 31

Effect of phentolamine on ADH release induced by hypertonic NaCl. The experimental protocol is the same as described in Fig. 29. Each point is the mean of 5 observations ± S.E.M.


N.S.
N.S.
15
10
5
0
ADH pg/min
Base
NaCl
(400 mOsm)
+ Phentol.
Recovery
Recovery