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).


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.
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.
Figure 8

Dose-response curve of nicotine-induced ADH release. Each point represents the mean of 6 observations ± S.E.M.
Figure 9

Effect of varying doses of nicotine 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.
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.
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.
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-11)

A-11, like McN-A-343-11 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-11 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-11 (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.
A. log-dose ng/kg/min body weight

Δ ADH pg/ml
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.
Figure 14
Dose-response curve of norepinephrine-induced ADH release in dogs.
Each point is the mean of 4 observations ± S.E.M.
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 \( \pm \) S.E.M.
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 ± S.E.M.
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.
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.
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 ± 8.15 pg/ml after hyperosmotic stimulation (3 M NaCl) alone. After hyperosmotic stimulation (3 M NaCl) propranolol and the change in ADH level was 12.36 ± 5.74 pg/ml (p > 0.05). The rise in plasma osmolality was similar before and after propranolol (14.4 ± 2.8, 16.0 ± 2.07; p > 0.05) (Fig. 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.
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 $1.38 \times 10^{-5}$ M caused an increase in ADH release from $17.33 \pm 5.40$ pg/min to $41.5 \pm 10.8$ pg/min, and then returned to basal level of $15.9 \pm 3.29$ 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, $6.9 \times 10^{-5}$ M also increased the release of ADH from $16.4 \pm 10.5$ pg/min to $27.7 \pm 13.7$ pg/min; but it was not significant statistically (p > 0.05).
Figure 20
Basal release of ADH by SONH during the experimental phase, as 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, represents 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 ± S.E.M.
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 ± S.E.M.
ADH pg/min

p < .05

Base
Ach
Recovery
2. Nicotine

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


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

4. Angiotensin-II (A-11)

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

5. Isoproterenol (IP)

IP, at a concentration of $2.37 \times 10^{-5}$ M had no effect. But, at higher concentration ($11.85 \times 10^{-5}$ 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 $1.48 \times 10^{-5}$ M and $7.38 \times 10^{-5}$ 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

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.
$p < .05$

<table>
<thead>
<tr>
<th>ADH pg/min</th>
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<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>20</td>
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</tbody>
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- **Base**
- **A. II**
- **Recovery**
Figure 2k
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.
ADH pg/min
N.S.
20
10 -
0 -
Base McN-A-343-I
Recovery

ADH pg/min
Base McN-A-343-I Recovery
N.S.

0 10 20

[Bar chart showing ADH pg/min for Base, McN-A-343-I, and Recovery, with N.S. indicating no significant difference]
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.
The diagram shows the levels of ADH (in pg/min) measured at different stages:

- **Base**
- **IP $1.18 \times 10^{-5} M$**
- **Recovery**

For ADH levels:

- **Base** level is indicated with a light shade.
- **IP $1.18 \times 10^{-5} M$** level is indicated with a dark shade.
- **Recovery** level is indicated with a light shade.

The levels are compared at these stages, showing no significant difference (N.S.) between the **Base** and **IP** stages, and similarly between the **IP** and **Recovery** stages.

For the ADH levels:

- **Base**: 20 pg/min
- **IP**: 30 pg/min
- **Recovery**: 20 pg/min

The diagram helps illustrate the effect of the treatment on the ADH levels over time.
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.
STIMULATION OF ADH RELEASE BY NOREPINEPHRINE (NE) IN SONH

$p < .05$

**ADH (pg/min)**

- Base NE Recovery
- $7.38 \times 10^{-5} \text{ M}$
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 \( 1.49 \times 10^{-3} \) 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 \( 4.60 \times 10^{-4} \) 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 ± S.E.M.
Osmolarity (mOsm/kg)

$r = 0.614$
$p < 0.05$

Osmolarity (mOsm/kg)
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 ± S.E.M.
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 $1.49 \times 10^{-3}$ 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.
Base + Mec.
NaCl Recovery (400 mOsm) + Mec.
Recovery
Recovery

N.S.
N.S.

ADH pg/min

Base
NaCl
Recovery
Recovery

0
5
10
15
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.
Base NaCl Recovery Recovery (400 mOsm) + C5

N.S. N.S.

ADH pg/min

15
10
5
0

Base NaCl Recovery Recovery (400 mOsm) + C5
3. Phentolamine

The α-blocking drug, phentolamine, at a concentration of $4.6 \times 10^{-4}$ 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.
Base NaCl Recovery (400 mOsm) + Phentol. Recovery

ADH pg/min

Base
NaCl (400 mOsm) + Phentol.
Recovery
Recovery

N.S.
N.S.