Sensory information from the kidney carried in afferent renal nerves (ARN) has been suggested to be of considerable importance in renorenal reflexes, the regulation of the circulation, and the pathogenesis of certain forms of experimental hypertension (8, 15, 25, 27, 29, 35, 42, 49). Afferent information from the kidney is thought to originate in several different classes of sensory receptors: renal mechanoreceptors sensitive to changes in arterial, venous, or ureteral pressure or mechanical stimuli (6, 31, 40, 47) and chemoreceptors activated by ischemia and changes in the ionic environment of the interstitium (34–37). There are also experimental data available suggesting that the kidney contains nociceptors (26, 40). Selective renal receptor activation (19, 35) or electrical stimulation of ARN (8, 10, 32) has been shown to elicit a variety of hemodynamic responses mediated by sympathetic nervous system and humoral mechanisms.

Methods

General procedures. Experiments were done in 36 adult male Wistar rats (250–400 g) anesthetized with pentobarbital sodium (Somnotol, MTC Pharmaceutical, Cambridge, ON, Canada; 50 mg/kg iv followed by supplemental doses of 2 mg/kg iv every 1–3 h). Laboratory rat chow was removed from the animals for ~12 h before the experiment to facilitate abdominal surgery. However, water was available ad libitum. All experimental procedures were done in accordance with the guidelines on the use and care of laboratory animals as set out by the Canadian Council on Animal Care and approved by the Animal Care Committee at the University of Western Ontario.

Polyethylene catheters (PE-50) were inserted into the femoral artery and vein for the recording of arterial pressure and the administration of drugs, respectively. Arterial pressure was recorded via a Statham pressure transducer (model P23 Db), and a Grass tachograph (model 7P4FG) that was triggered by the arterial pressure pulse was used to monitor...
heart rate. Both arterial pressure and heart rate were recorded continuously on a Grass polygraph (model 79D). The trachea was cannulated, and the animals were allowed either to breathe spontaneously or were artificially ventilated using a small rodent ventilator (model 683, Harvard Apparatus) with a mixture of room air and 95% O2. During the course of the single-unit recording experiment, the depth of anesthesia was continually monitored by examining withdrawal reflexes. Body temperature was monitored and maintained at 37 ± 1°C by a heating pad controlled by a temperature controller (model 73A; Yellow Springs Instruments).

Isolation of ARN. The ARN of the left renal plexus were isolated as previously described (18) in 20 animals. In brief, after a midline abdominal incision, the left renal nerves were carefully dissected free from the hilus of the kidney for ~1 cm and placed on a bipolar stainless steel hook electrode. The identity of the isolated renal nerves was confirmed by the pronounced blanching of the kidney during electrical stimulation (20 Hz frequency, 0.5–1.0 ms pulse duration, 10-s pulse train, 250–500 µA stimulus intensity). The stimulus was delivered from a Grass S88 stimulator through a Grass PSIU 6D stimulus isolation and constant current unit. The renal nerves were then crushed distal to the stimulating electrode to eliminate the possibility that stimulation of the renal nerves may induce cardiovascular changes associated with the release of renin (20). The renal nerves and stimulating electrode were covered with cotton pellets soaked in warm Dow Corning 360 medical fluid (Midland, MI) to ensure electrical isolation and to prevent the drying of nervous tissue. The renal nerves were again electrically stimulated (20 Hz, 0.5–1.0 ms pulse duration, 10-s pulse train) to determine the threshold intensity of the stimulus required to elicit an arterial pressure response (250–500 µA).

Renal venous or arterial occlusion. As previously described (19), in all experiments (n = 16) the left kidney was exposed through a midline abdominal incision. Short segments (~ 5 mm) of the renal nerves, artery, and vein were carefully cleared of connective tissue 5–10 mm from the hilus of the kidney and silk threads were placed around the renal nerves, artery, and vein to allow the later occlusion of the artery and/or vein by retraction of the threads and the transection of the renal vessels. After completion of the renal surgical region of the abdominal cavity around the exposed renal nerves and blood vessels was filled with warm Dow Corning 360 medical fluid (Midland, MI). The surgical incision was maintained open to allow for visual confirmation of subsequent renovascular occlusion. RVO or RAO was done by retracting the silk ligature around the renal vein or artery, respectively. RVO, which has been shown to activate renal mechanoreceptors (6, 8, 23, 37), was maintained usually for 60–150 s. RAO was used to activate R1 and R2 renal chemoreceptors (36, 37). RAO produced blanching of the kidney, which was taken to indicate an ischemic state. As R2 chemoreceptors have been shown to display an onset latency of ~35 s in response to ischemia (36, 37), the period of RAO in these studies was 100 s.

Recording of PVH neurosecretory neurons during ARN and renal receptor stimulation. The animal was placed in the supine position, and the head was fixed in a Kopf stereotaxic frame. The ventral surface of the hypothalamus and of the hypophysis were exposed using a transphenoidal approach (18, 19). The exposed area of the ventral surface of the brain extended from the rostral aspect of the optic chiasma caudally to the caudal extent of the hypophysis. A bipolar, stainless steel stimulating electrode (SNEX-100; 0.25 mm tip diameter; 0.25 mm tip-to-concentric ring distance; 50–60 K3 initial resistance in saline; David Kopf, Tujunga, CA) was placed in the neurohypophysis or in the neurohypophysial stalk to antidromically activate PVH neurons projecting to the neurohypophysis. Neurons encountered during an electrode penetration were assessed for antidiromic activation using previously established criteria (28): constant latency of the evoked spike, high following frequency of the evoked spike, and cancellation of the evoked spike with a spontaneous evoked spike. The stimulus applied to antidromically activate neurons in the PVH was a rectangular pulse of 1-ms pulse duration, 0.5–1 mA stimulus intensity delivered at 1 Hz from a Grass S88 stimulator through a Grass PSIU 6D stimulus isolation and constant current unit (13, 14). The region of the PVH was systematically explored for spontaneously active single units recorded extracellularly using glass microelectrodes (4–15 MΩ impedance, 1–3 µm tip diameter) filled with 2% Pontamine sky blue in 0.5 M sodium acetate. Electrode penetrations through the region of the PVH were made on a grid pattern with points ~300 µm apart. Single-unit activity was amplified through a multipurpose microelectrode amplifier (Axoprobe-1A, Axon Instruments, Foster City, CA) and displayed on a Tektronix R5113 oscilloscope for observation and photography. The action potentials were also discriminated by a slope/height window discriminator (Frederick Haer Company, Brunswick, ME), and peristimulus time histograms were generated using either an AST 286 computer (analog-to-digital conversion board from Data Translation, Marlboro, MA) or a Neurograph STA-1 microprocessor (13, 18, 19).

Magnocellular PVH neurons projecting to the neurohypophysis were classified as either putative AVP or putative Oxy neurons on the basis of their discharge patterns and their response to baroreceptor activation. In the rat, AVP neurosecretory neurons exhibit either a continuous or phasic discharge pattern and Oxy neurosecretory neurons generally discharge continuously (33). Therefore, PVH neurons that projected to the neurohypophysis, were either continuously or phasically discharging, and were reflexively inhibited by an acute increase in arterial pressure (phenylephrine HCl; Sigma, St. Louis, MO; 2–10 µg iv) were classified as putative AVP neurons (33). Continuously active PVH neurons projecting to the neurohypophysis and that were unresponsive to baroreceptor activation were classified as putative Oxy neurons. Baroreceptor activation was confirmed by the reflex decrease in heart rate that was elicited during the rise in arterial pressure after the administration of the phenylephrine HCl.

Putative AVP and Oxy neurons in both the ipsilateral and contralateral PVH were tested for their response to electrical stimulation of the ARN by applying a stimulus (3- to 5-pulse train at 200 Hz, 0.5- to 1-ms pulse duration, 250–750 µA stimulus intensity) (18) once every second to the isolated ARN. Application of this stimulus to the ARN did not elicit changes in systemic arterial pressure. Peristimulus time histograms of evoked responses from putative AVP or Oxy neurons to ARN stimulation were generated by summing the evoked responses over 100–300 superimposed sweeps (2–4 ms/bin).

Histological localization of recording sites and immunohistochemical identification of AVP and Oxy neurons in PVH. Most sites of recording and all stimulation sites were marked at the end of each experiment. Recording sites were also determined by interpolation from Pontamine sky blue deposits [4–5 µA cathodal direct current (DC) for 15–30 min] in an electrode penetration. Stimulation sites were marked by depositing iron from the electrode tip (20–30 µA anodal DC current for 20–30 s). The animals were perfused with 50 ml of 0.9% saline solution followed by 50 ml of 1% potassium.
ferrocyanide in 10% buffered Formalin to reveal the marked stimulation sites by the Prussian blue reaction. The brains were postfixed in the buffered Formalin for 2–4 days. Frozen transverse sections at 50 µm were cut in a cryostat, mounted on glass slides, and stained with neutral red. Recording sites were mapped on projection drawings of the forebrain from each animal. The nomenclature from Swanson and Kuypers (46) of the PVH was used.

Immunohistochemistry was done in some of the animals (n = 6) in which the renal vein or artery were occluded to show the location of the recording sites in relation to AVP- and Oxy-immunoreactive neuronal perikarya in the PVH using methods previously described (11). Briefly, the anesthetized animal was perfused transcardially with 300 ml of saline followed by 500 ml of Zamboni’s fixative (2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2; 15% saturated picric acid). The brain was removed and then placed in Zamboni’s fixative. At ~24-h intervals, the brain was transferred sequentially to 5 and 10% sucrose-PBS solutions at 4°C. Frozen, serial transverse sections of the forebrain through the region of PVH were cut on a cryostat (~20°C, Bright Instrument) at a thickness of 40 µm, and alternate sections were collected in PBS for immunocytochemical processing of AVP- or Oxy-immunoreactive neuronal perikarya. Tissue sections were processed for 30 min in normal goat serum (Vector Laboratories, Burlingame, CA) diluted 1:50 in PBS containing 0.3% Triton X-100 (Sigma). After two washes in PBS, the sections were placed for 16–20 h at 4°C in primary rabbit antiserum (Immuno Nuclear, Stillwater, MN) diluted 1:1,000 in PBS-Triton X-100. The possibility of crossreactivity of the AVP antiserum with Oxy was minimized by preabsorption of the diluted antiserum with 10 µg/ml of synthetic Oxy (11). Similarly, the diluted Oxy antiserum was preabsorbed with 10 µg/ml of synthetic AVP (11). The sections were then washed in PBS and placed for 30 min in rabbit biotinylated IgG antibody (Vector Laboratories) diluted 1:200 in PBS-Triton X-100. After a wash in PBS, the sections were placed in a solution containing methanol and hydrogen peroxide. Two washes in PBS were followed by placement of the sections for 90 min in avidin-biotin complex (ABC) reagent (Vector Laboratories) in PBS-Triton X-100. Sections were washed in PBS, and the horseradish peroxidase contained in the ABC complex was visualized by placing the sections for 10–30 min in 0.006% hydrogen peroxide and 0.02% 3,3’-diaminobenzidine (Sigma) in PBS. Sections were rinsed in PBS and mounted onto glass microscope slides, dried, and stained with neutral red to visualize the cytoarchitectural boundaries of hypothalamic structures. A Leitz Diaplan light microscope was used for examination and photography of the tissue sections. Sites of recording from all animals were mapped on projection drawings of transverse sections through the PVH region and matched to the distribution of AVP- or Oxy-immunoreactive neuronal perikarya. In this manner, sites of recordings of putative AVP and Oxy neurons that projected to the neurohypophysis and that were tested for their response to ARN stimulation could be shown to be contained within the boundaries of the magnocellular regions of PVH containing AVP- or Oxy-immunoreactive neuronal perikarya.

Data analysis. The latency of the evoked antidromic action potentials was taken as the time interval between the stimulus artifact and the beginning of the rising phase of the extracellularly recorded action potential. To determine the responses of putative AVP and Oxy neurons in PVH to electrical stimulation of ARN, the criteria used to determine whether a single unit responded orthodromically were similar to those previously described (13, 18, 19). In brief, changes in the discharge rate of neurons to stimulation of ARN were identified and quantified by comparing the height of each poststimulus bin of the peristimulus time histogram (PSTH) with the average bin height for the period of 100 ms before the stimulus (baseline activity). The onset latency of an orthodromic response was determined from the PSTH as the time interval from the stimulus artifact to the first of three consecutive bins with heights that were more than one standard deviation from the mean baseline discharge rate. The boundaries of possible periods of significant responses were defined as the occurrence of a period of time following the stimulation during which the mean height of the PSTH was at least 30% above or below the mean baseline discharge rate.

The discharge rates of units were also monitored during the acute rise in arterial pressure after the intravenous administration of phenylephrine and after renal venous or arterial occlusion. In these cases, a running ratemeter record of the discharge rate of the unit during the administration of the drugs or during vessel occlusion was compared with the baseline discharge rate before the drug injection or vessel occlusion. A mean bin height of at least 30% above or below the mean baseline discharge rate (calculated from the preceding 100 s) for at least three consecutive bins was accepted as a significant response. Subsequent changes in discharge rates after the application of a stimulus were expressed as a percentage of the control discharge rate. All units were tested for their response to baroreceptor activation, RAO, or RVO at least twice to determine the reproducibility of the response of the neuron to the stimulus.

Results are expressed as means ± SE. Response latencies and durations, conduction velocities, and spontaneous discharge rates were compared statistically using Student’s t-test. A P value of <0.05 was considered to indicate statistical significance.

RESULTS

Identification of putative AVP and Oxy neurons in PVH. A total of 143 neurons within the PVH region and 4 ventrolateral to the PVH in the lateral hypothalamic area was antidromically activated by stimulation of the neurohypophysis. Of these neurons, 73 were classified as putative AVP and 70 as putative Oxy neurons. AVP neurons responded with a mean antidromic latency of 18.9 ± 1.4 ms. This latency was not different from that of Oxy neurons (17.9 ± 1.3 ms). The combined latency of AVP and Oxy neurons (18.4 ± 1.4 ms) corresponded to an estimated conduction velocity of ~0.14 m/s for PVH-neurohypophysis projecting axons. These latencies and axonal conduction velocities are similar to those previously reported for nonmyelinated hypothalamo-neurohypophysis axons (33).

AVP neurons were found to discharge spontaneously (5.6 ± 0.7 spikes/s) in either a slow, irregular pattern (n = 40); fast, continuous pattern (n = 21); or a phasic pattern (n = 12) (33). In contrast, Oxy neurons discharged spontaneously (3.4 ± 0.7 spikes/s) in a slow, continuous pattern at rates significantly slower than those of the AVP neurons. The spontaneous discharge of all AVP neurons was inhibited during the reflex activation of baroreceptors, whereas that of Oxy neurons was not altered (Fig. 1, A and B).

Response of putative AVP and Oxy neurons to ARN stimulation. Ninety-eight neurohypophysial projecting neurons histologically verified in the PVH were tested
for their response to electrical stimulation of ARN (Table 1, Figs. 2 and 3). Of these, 44 were classified as putative AVP and 54 as putative Oxy neurons (Table 1). Most (89.5%; 43/48) of the putative neurosecretory neurons responding to ARN stimulation (17 AVP and 26 Oxy neurons) (Fig. 1C) were found within the contralateral PVH (Table 1). Although a small number (n = 5) of the AVP neurons were also found to be excited by ARN stimulation in the ipsilateral PVH, no Oxy neurons were found to receive ARN inputs in the ipsilateral PVH. Of the 22 AVP neurons that responded to ARN stimulation, most (20/22) were excited and only two were found to be inhibited by ARN. A representative example of an AVP neuron within the PVH that responded to ARN stimulation is shown in Fig. 1. The mean onset latency of the response of the AVP neurons to ARN stimulation was 162 ± 16 ms, and the duration of the ARN response was 148 ± 19 ms.

Of the 26 Oxy neurons responding to ARN stimulation, 23 were excited and 3 were inhibited by ARN inputs. An example of a putative Oxy neuron excited by ARN stimulation is shown in Fig. 1. The mean onset latency of the response of putative Oxy neurons to ARN stimulation was 152 ± 10 ms, and the mean duration of the ARN response was 130 ± 11 ms.

No significant differences were found in the response latencies and the response durations to ARN stimulation between putative AVP and Oxy neurons. In addition, a similar percentage of AVP (44.9%) and Oxy (55.1%) neurons were found to respond in the PVH to stimulation of ARN (Table 1).

An additional four putative neurosecretory neurons were identified in the lateral hypothalamic area just ventrolateral to the PVH. These neurons appeared to be part of the nucleus circularis. None of these neurons responded to baroreceptor stimulation, suggesting that they were putative Oxy neurons. These neurons did not respond to stimulation of ARN.

Responses of putative AVP and Oxy neurons to renal venous or arterial occlusion. Forty-five putative magnocellular neurosecretory neurons (29 AVP and 16 Oxy neurons) were tested for their response to renal vessel occlusion in the contralateral PVH (Table 2). Twelve AVP neurons and two Oxy neurons were found to be inhibited by RVO. In addition, no AVP or Oxy neurons were found to be excited by RVO (Table 2). The latency of the inhibition of AVP neurons to RVO was found to be 21 ± 7 s, and the duration of the inhibition was 97 ±

Table 1. Putative AVP and Oxy neurons in the PVH that responded to ARN stimulation

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Excited</th>
<th>Inhibited</th>
<th>No Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putative AVP neurons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>29</td>
<td>15</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Putative Oxy neurons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>34</td>
<td>23</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

Note that a greater number of arginine vasopressin (AVP) and oxytocin (Oxy) neurons responded to afferent renal nerve (ARN) stimulation in the contralateral paraventricular nucleus of the hypothalamus (PVH) compared with the ipsilateral PVH. In addition, a greater number of AVP neurons was excited by ARN stimulation compared to Oxy neurons.
19 s. The neurons decreased their discharge rate by 77 ± 11% of control. The remaining 17 AVP and the 14 Oxy neurons tested did not respond to RVO (Table 2). An example of an AVP neuron responding with inhibition to RVO is shown in Fig. 4.

RAO elicited an increase in the discharge rate of 8 of 24 (33%) of the AVP neurons tested and an increase in the discharge of 1 of 11 (9%) of Oxy neurons tested (Table 2). The remaining AVP and Oxy neurons tested for their response to RAO did not alter their discharge rate to the stimulus. No neurons were found to be inhibited by RAO. The increase in activity in the eight AVP cells that were excited by RAO was 140 ± 45% above control discharge rates. The mean latency of the response of AVP neurons to RAO and the mean duration of this excitatory response were 37 ± 18 and 69 ± 9 s, respectively. A representative example of an AVP neuron excited by RAO is shown in Fig. 4.

The eight putative AVP neurons found to respond with an increase in discharge rate to RAO were also tested for their response to RVO. All eight neurons were also found to be inhibited during RVO (Fig. 4).

To investigate whether the response of putative AVP neurons to RAO was due to activation of renal receptors and not changes in systemic arterial pressure, the effect of ARN transection on the response of four AVP neurons that responded to RAO was investigated. In all cases the response of the neuron to RAO was abolished after ARN transection.

Cardiovascular responses to RVO or RAO. In most cases when the renal vein was occluded, a decrease (−11 ± 1 mmHg) in mean arterial pressure (MAP) was observed similar to that reported previously (3, 13, 14). However, on three occasions RVO elicited a small pressor (MAP, +6 ± 1 mmHg) response. Regardless of the direction of the arterial pressure response to RVO, as described above, the AVP or Oxy units responded with inhibition only. RAO exclusively resulted in a pressor (MAP, +7 ± 1 mmHg) response as reported previously (14). However, on occasion a transient depressor (MAP, −8 ± 2 mmHg) response followed the pressor response during RAO. It was also observed that the direction of the changes in arterial pressure during RAO did not alter the excitatory response evoked in the AVP neurons (Fig. 4).

Fig. 2. Transverse sections of the PVH [extending from approximately 7.0 (C) to 7.4 (A) mm rostral to the intra-aural line] taken at 3 representative rostrocaudal levels. Each section drawn is separated by −0.2 mm. Photomicrographs of the PVH (left) show the distribution of AVP immunoreactive neurons in the magnocellular and parvocellular regions of the PVH. Schematic drawings of the PVH on the right correspond to the mirror images of the photomicrographs on the left and show the locations of putative AVP neurons tested for their response to ARN stimulation or occlusion of the renal of the renal vessels. ●, △, Putative AVP neurons responding to electrical stimulation of ARN with excitation or inhibition, respectively. ○, Nonresponsive neurons to electrical stimulation of ARN or to renal vein occlusion (RVO) or renal artery occlusion (RAO). ●, Putative AVP neurons excited by RAO and inhibited by RVO; ●, putative AVP neurons inhibited by RVO. AHA, anterior hypothalamic area; dp, dorsal parvocellular component of the PVH; mp, medial parvocellular component of the PVH; pm, magnocellular component of the PVH; pv, periventricular nucleus of the PVH; v, 3rd ventricle. At left, photomicrographs show blood vessels (+). Calibration mark in photomicrograph C indicates 100 µm.
magnocellular components of the PVH containing immunoreactive neurons to AVP. Putative AVP neurons tested for their response to electrical stimulation of ARN or renal vessel occlusion. Note that the photomicrographs A-C are the adjacent sections to those shown in Fig. 2, A-C, respectively. ○, Putative Oxy neurons in the PVH that were either excited or inhibited by electrical stimulation of ARN, respectively. ▲, Nonresponsive neurons to electrical stimulation of ARN or renal vessel occlusion. ●, Putative Oxy neurons inhibited by RVO. ▲, Putative Oxy neuron excited by RAO. See Fig. 2 for additional details. Calibration mark in photomicrograph C represents 100 µm.

Table 2. Percentage of putative AVP and Oxy neurons within the contralateral PVH that responded to RVO or RAO

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>AVP Neurons</th>
<th>Oxy Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Excited</td>
</tr>
<tr>
<td>RAO</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>RVO</td>
<td>29</td>
<td>0</td>
</tr>
</tbody>
</table>

All neurons tested for renal artery occlusion (RAO) were also tested with renal vein occlusion (RVO). ●, Excited; ▲, inhibited; NR, nonresponsive. n, Number of neurons tested. Note that RVO only inhibited AVP and Oxy neurons and RAO exerted only an excitatory effect on AVP and an inhibitory effect on Oxy neurons.
these neuronal populations were antidromically activated by stimulation of the neurohypophysis, it is likely that they function as neurosecretory neurons. Electrical stimulation of ARN or stimulation of renal receptors by occlusion of the renal vessels evoked a change in the discharge rate of ~42% of the putative AVP neurons and of ~23% of the putative Oxy neurons tested in the PVH. The finding that neurons in the PVH receive ARN inputs is consistent with previous findings in the cat (10, 14, 15) and the recent demonstration that ARN stimulation induces c-Fos expression in the PVH of the rat (42). In addition, the finding that both AVP and Oxy neurons in the PVH receive ARN inputs is supported by the observation that electrical stimulation of ARN in the conscious rat results in an increase in the release of AVP and Oxy into the systemic circulation (39). Similarly, electrical stimulation of ARN in the cat results in an increase in the plasma level of AVP (9). However, it has previously been shown that Oxy neurons in the SON do not respond to activation of ARN (18, 19). Taken together, these observations suggest that magnocellular neurosecretory neurons in the PVH or in accessory magnocellular nuclei may be involved in mediating the release of Oxy in addition to AVP and Oxy into the systemic circulation (39). In addition, these data suggest that magnocellular neurosecretory neurons in the PVH and the SON may be under different physiological control mechanisms and that ARN may contribute a differential input to these two hypothalamic nuclei. This suggestion is consistent with the finding that various stimuli result in a differential, but simultaneous, release of AVP and Oxy (24). In addition, it was found in this study that AVP and Oxy neurons within the PVH are not stimulated by the nonspecific activation of afferent renal fibers but appear to respond selectively and differentially to activation of putative renal mechanoreceptors or renal chemoreceptors after RVO or RAO, respectively.

It is of interest that the few putative Oxy neurons recorded in this study in the nucleus circularis, an accessory magnocellular neuronal group in the hypothalamus, did not respond to ARN stimulation. This finding is consistent with the observation that accessory magnocellular nuclei lack Fos-labeled neurons after ARN stimulation in the rat (42). This suggests that the PVH is likely solely responsible for the release of Oxy during stimulation of ARN in the rat. However, ARN input to both AVP and Oxy neurons within the PVH may also be species specific, as ARN stimulation in the cat has been shown to activate putative AVP and not Oxy neurons in the PVH (10).

It may be argued that the responses of the putative AVP and Oxy neurons in the PVH were due to the changes in systemic arterial pressure that occurred during RVO or RAO. This possibility was considered unlikely, as neurons responded in one direction although the arterial pressure responses elicited by RVO or RAO were variable in magnitude and sometimes in direction. In addition, it would be expected, especially for the AVP neurons, that whenever the arterial pressure increased, the discharge rate of the neurons would decrease as observed during the activation of baroreceptors. However, this did not occur. Finally, it was shown in a few recordings from AVP neurons that transection of the renal nerves abolished response of the neurons to RAO.

The latency of the response in PVH neurons to stimulation of ARN was similar to that observed for AVP neurons in the SON (18). Therefore, as previously suggested (18), these latencies correspond to an average overall afferent pathway conduction velocity of <1 m/s. Although the conduction velocities of axons in the
central pathways that carry information to the hypothalamus are not known, this observation suggests that the pathway from the kidney to the PVH may be composed primarily of neurons with unmyelinated axons. Electrophysiological studies have shown that ARN fibers that project to the spinal cord can be divided into at least two groups: slow-conducting fibers composed of both unmyelinated and thinly myelinated axons and more rapidly conducting, small myelinated fibers (26). If the suggestion is accepted that unmyelinated afferent fibers were predominantly stimulated in this study, then most of the inputs that reach the PVH likely mediate chemoreceptor information, as chemoreceptor and not mechanoreceptor afferent information is thought to be carried by unmyelinated afferent fibers (34–37).

The central pathways that mediate the renal afferent information to the hypothalamus have not been elucidated (15). However, it is known that afferent renal inputs alter the activity of spinoreticular and spinothalamic projecting neurons (1–3, 43, 48). It is not unreasonable to suggest that these neurons in turn activate PVH neurons, as both brain stem and thalamic nuclei are known to provide extensive inputs to the hypothalamus. The most likely pathway may involve a direct projection from the spinal cord to the ventrolateral medulla (48). This region of the brain stem is known to have projections that directly innervate magnocellular neurosecretory neurons in the PVH and the SON (for reviews, see Refs. 15, 16). This suggestion is supported by the observation that the pathway from the ventrolateral medulla to the hypothalamus is primarily catecholaminergic (for review, see Ref. 16), and renal denervation has been shown to alter hypothalamic catecholamines (7).

In the present study, putative AVP neurons were predominantly excited by the renal ischemia produced by RAO. The renal ischemia is known to activate both R1 and R2 renal chemoreceptors (36, 37). Day and Ciriello (19) did not find AVP neurons in the SON that respond to RAO. It was suggested that the central pathways mediating this afferent information to the hypothalamus may have been affected by the level of anesthesia. This has also been suggested previously to account for the varying cardiovascular effects observed during stimulation of ARN (8). Although this possibility cannot be completely eliminated, it appears unlikely, as the same anesthetic was used in the present study and excitatory responses to RAO were observed in AVP PVH neurons.

Stimulation of renal mechanoreceptors by RVO was successful in inhibiting the discharge of a small population of putative AVP neurons tested. This is also in contrast to a previous study in which it was found that RVO was not capable of eliciting a response from putative AVP neurons in the SON (19). The present findings, taken together with those of Day and Ciriello (19), support the suggestion that AVP can be released by the activation of pathways that selectively alter the activity of specific populations of hypothalamic nuclei and that there is a differential distribution of renal sensory input to the hypothalamus.

Perspectives

It is interesting to note that RAO is a powerful stimulus for the release of renin from the kidney. The resultant activation of the renin-angiotensin II system would lead to an increase in the plasma level of AVP and also result in vasoconstriction. The excitatory input to AVP neurons may function to potentiate the release of AVP. This in turn would further augment the already existing vasoconstriction and renal ischemia and create a positive feedback loop that could potentially lead to a sustained increase in systemic arterial pressure in an attempt by the kidney to increase its perfusion pressure. In support of this suggestion, it was recently shown that electrical stimulation of ARN in the rat excites neurons in the subfornical organ that project to the PVH and that respond to systemic changes in angiotensin II (13). On the other hand, the activation of mechanoreceptors in the kidney by the increased pressure would be expected to decrease mechanisms that may contribute to the elevated arterial pressure. Therefore, it would be expected that the discharge rate of putative AVP neurons would be decreased to prevent the release of AVP into the circulation. It is possible that mechanoreceptor and chemoreceptor input from the kidney form an important regulatory mechanism for the release of AVP. It has been suggested that a balance between cardiovascular and renal afferent input to supraspinal structures exists and that a physiological or pathological shift in the balance favoring the expression of renal chemoreceptor inputs may contribute to the development and/or maintenance of hypertension by providing an excitatory drive to sympathetic and neurohumoral systems (15). It is therefore not surprising that neurons in the PVH receive this inhibitory input, as the PVH has been implicated in the development of hypertension in several different experimental models (12, 17, 50). In addition, it is generally considered that the PVH has a greater connectivity with other neural areas involved in autonomic functions, including the cardiovascular-associated areas of the brain stem and forebrain, compared with the connections of the SON.

The physiological significance of ARN input to Oxy neurons within the PVH is not known. Oxy release has been shown to be influenced by changes in plasma osmolality (4, 5, 30), and Oxy binding sites have been identified in the kidney (38, 45). As R2 chemoreceptors appear to function as renal osmoreceptors (34), it is not unreasonable to suggest that activation of ARN fibers carrying osmoreceptor information from the kidney activated some of the putative Oxy neurons within the PVH. PVH Oxy neurons have been implicated in body fluid balance, as stimulation of the subfornical organ with angiotensin II results in an increase in the release of Oxy into the circulation (21). In addition, stimulation of the subfornical organ activates PVH Oxy neurons (22) and systemic angiotensin has been shown to act through the subfornical organ to activate Oxy neurons.
within the PVH (22). Simon et al. (39) previously showed that ARN stimulation also increases plasma Oxy levels. It is therefore possible that ARN input may influence the activity of Oxy neurons in the PVH as part of the physiological responses to osmotic changes that are sensed as blood and/or filtrate pass through the kidney.

In summary, these data demonstrate that stimulation of ARN alters the activity of both AVP and Oxy neurons in the PVH, which likely results in the increased plasma AVP and Oxy levels during ARN stimulation (39). These data also provide evidence of the nature of this renal sensory input to AVP and Oxy neurons, however, remains to be fully determined. The results also suggest that ARN inputs to PVH neurons likely contribute to the regulation of arterial pressure and body fluid balance. Finally, these data further support the suggestion that PVH magnocellular neurosecretory neurons may be involved in homeostatic mechanisms different from those during which the SON neurons are activated.

The contribution of T.-X. Zhang and J. K. Simon to the electrophysiological studies and of J. A. Nichols to the histological processing of the brain tissue is gratefully acknowledged. The author thanks Dr. L. P. Solano-Flores for valuable comments during the preparation of this manuscript.

This work was supported by the Heart and Stroke Foundation of Ontario.

Address reprint requests to J. Ciriello.

Received 23 September 1997; accepted in final form 30 July 1998.

REFERENCES


