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EXCITATION OF NEURONES IN THE RAT PARAVENTRICULAR NUCLEUS *IN VITRO* BY VASOPRESSIN AND OXYTOCIN

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SUMMARY

1. Extracellular recordings were made from ninety-seven spontaneously firing cells in the paraventricular nucleus (p.v.n.) of the rat hypothalamic slice preparation.

2. The spontaneously firing cells tested fired at 0·1–8 spikes/s but the majority showed a slow irregular firing pattern. The average firing rate of all ninety-seven cells was 2·2 ± 0·2 spikes/s (mean ± S.E. of mean). Six cells showed a phasic firing pattern.

3. Following bath application of arginine-vasopressin (AVP) 10^{-7} M, sixty-four (66%) of ninety-seven p.v.n. cells showed excitatory responses and three (3%) cells inhibitory responses. Bath application of oxytocin (OXT) 10^{-7} M excited thirty-nine (57%) of sixty-eight p.v.n. cells and inhibited two (3%) cells. Individual p.v.n. cells responded to application of both AVP and OXT, but the magnitude and threshold of the responses varied from cell to cell. Of the sixty-six cells tested with both peptides at 10^{-7} M, sixteen showed similar responses to both and fifteen showed no response to either: twenty cells showed a greater response to AVP and fifteen a greater response to OXT. Of six phasic firing cells, two showed excitatory responses to AVP and all four cells tested did not show any response to OXT.

4. The dose-dependence of the response to AVP and OXT was tested in six p.v.n. cells. There was a direct relationship between peptide concentration and increased firing rate. The threshold concentration of the peptides ranged from 10^{-8} to 10^{-10} M.

5. The cells responsive to the peptides were not located in particular areas of the p.v.n. but were diffusely distributed throughout the nucleus.

6. After blocking synaptic transmission with a low Ca^{2+} and high Mg^{2+} medium, all tested cells (AVP, $n = 15$; OXT, $n = 14$) which had responded to applications of AVP or OXT in normal medium still showed responses to the peptides, although the effect was less marked in half the cells. However, in the absence of synaptic transmission two cells showed unimpaired responses to one of the peptides but greatly depressed responses to the other.

7. The V_1-receptor antagonist [1-(β-mercapto-, β-cyclopentamethylenepropionic acid)], 8-D-arginine-vasopressin (d$(CH_2)_5$DAVP) or V_1/V_2-receptor antagonist [1-(β-mercapto-, β-cyclopentamethylenepropionic acid), 2-D-tyrosine,4-valine]arginine-vasopressin (d$(CH_2)_5$D-TyrVAVP) completely or partly blocked the AVP-induced responses, while the V_2-receptor agonist 1-deamino-8-D-arginine-vasopressin (dDAVP) did not influence the spontaneous discharges of the cells.

8. To determine whether the peptides excited all hypothalmic cells non-selectively, the effects of AVP and OXT on twenty cells in the suprachiasmatic nucleus (s.c.n.) were examined. AVP excited four cells and OXT four cells.

9. We conclude that p.v.n. cells were sensitive to AVP and OXT, probably through specific receptors for each peptide: for AVP, the receptor was probably of the V_1 type. These findings suggest that AVP and OXT may be functioning as neurotransmitters or neuromodulators in the p.v.n.

INTRODUCTION

The two peptides, arginine-vasopressin (AVP) and oxytocin (OXT) are well known as the classical posterior pituitary hormones which control body fluid balance and milk ejection. The peptides are mainly synthesized in neurones of the supraoptic nucleus (s.o.n.) and paraventricular nucleus (p.v.n.) and are released from axonal terminals located in the neurohypophysis.

Recent immunocytochemical studies have shown that neurones located in the s.o.n. and p.v.n. which contain the peptides also send processes to extrahypothalamic areas (Buijs, 1980; Kawata, Ueda & Sano, 1983) and to neighbouring neurones within the s.o.n. and p.v.n. (Sofroniew & Glasmann, 1981) as well as to the neurohypophysis and to the median eminence. The effects of application of AVP and OXT to single neurones have been investigated in several areas of the central nervous system (C.N.S.), for example the medulla (Charpak, Armstrong, Mühlethaler & Dreifuss, 1984; Morris, Farmery, Roberts & Hill, 1984), hippocampus (Mühlethaler, Dreifuss & Gähwiler, 1982; Mühlethaler, Sawyer, Manning & Dreifuss, 1983), and spinal cord (Suzue, Yanaihara & Otsuka, 1981; Gilbey, Coote, Fleetwood-Walker & Peterson, 1982). Inside the s.o.n. and p.v.n., neurosecretory cells were also affected by application of AVP and OXT and by application of their analogue, lysine-vasopressin (Nicoll & Barker, 1971; Moss, Dyball & Cross, 1972; Leng & Mason, 1982). Release of the peptides in the septum and the nucleus tractus solitarius (Buijs & Heerikhuize, 1982) and in or near the s.o.n. and the p.v.n. (Chapmann, Hatton, Ho, Mason & Robinson, 1983; Moos, Freund-Mercier, Guerné, Guerné, Stoeckel & Richard, 1984) was observed in slice preparations. Moreover, there is a high density of AVP-binding sites in these regions (Van Leeuwen & Wolters, 1983; Biegon, Terlou, Voorhuis & De Kloet, 1984). Mühlethaler *et al.* (1982, 1983) found that AVP- and OXT-induced electrophysiological responses in the hippocampus could be blocked by their antagonists and receptors for vasopressin and OXT have also been investigated electrophysiologically in the s.o.n. (Abe, Inoue, Matsuo & Ogata, 1983). The previous results suggested that the peptides may act as neurotransmitters or neuromodulators in the s.o.n. and p.v.n. as well as in the other regions of the C.N.S.

However, the p.v.n. does not only contain neurosecretory cells but also is now regarded as an integrative site for neuroendocrine and autonomic functions (Swanson & Sawchenko, 1983). Since the p.v.n. is known to be innervated by terminals which contain a number of different peptides (Swanson & Sawchenko, 1983), it is an appropriate site to investigate the action of centrally acting peptides.

The aim of the present study was to investigate the effects of AVP and OXT on p.v.n. neurones studying the distribution of peptide-responsive neurones inside the

nucleus and to determine whether the effects were mediated by specific peptide receptors on the p.v.n. cells using the rat hypothalamic slice preparation.

METHODS

Tissue preparation

Adult male Wistar rats weighing 150–350 g were stunned by a blow on the neck and decapitated. The brains were then quickly removed and after cooling in bathing medium at 4 °C for approximately 1 min the meninges were removed under microscopic control. Coronal slices 350–450 μm in thickness were cut from a block of the brain tissue immersed in bathing medium at 4 °C using a vibratome-type slicer (Inenaga & Yamashita, 1983). Three or four slices of hypothalamic tissue were usually obtained from each brain. Immediately after sectioning the slices were placed in bathing medium at room temperature and left for at least 1 h until they were transferred to a recording chamber. Hypothalamic slices could be kept in good condition in the bath for periods up to 12 h. Prior to recordings, the slices were carefully trimmed with a microsurgery knife so that recordings were made from a piece of tissue containing the p.v.n. area with a total area less than 2 × 2 mm. Usually, a part of the anterior hypothalamus, zona incerta and fornix were included in the slices, as shown schematically in Fig. 3. The slices containing the suprachiasmatic nucleus (s.c.n.) were also trimmed so that the pieces of tissue were as small as those containing the p.v.n. The bathing and perfusing medium for slices was a modified Yamamoto solution which contained (in mM): NaCl, 124; KCl, 5; KH_2PO_4, 1·24; $MgSO_4$, 1·3; $CaCl_2$, 2·1; $NaHCO_3$, 20; glucose, 10 (Yamashita, Inenaga, Kawata & Sano, 1983). When required synaptic activity was blocked by using a perfusion medium in which calcium and magnesium concentrations were altered (i.e. $CaCl_2$, 0·5 mM and $MgSO_4$, 9 mM). This solution is referred to as a low Ca^{2+} and high Mg^{2+} medium. The solutions were oxygenated with a mixture of 95 % O_2 and 5 % CO_2 and kept at 36–37 °C by a water jacket.

The recording chamber was made of acrylic resin and had a volume of 0·8 ml. The slice was placed on a sylgard mat glued to the bottom of the chamber and held in place by a nylon net and platinum weights. The perfusion system was gravity-fed and had a dead space of 0·7 ml. The flow rate of perfusion medium in the recording chamber was adjusted to 3·0–5·0 ml/min. It could be completely exchanged in 30–45 s and was removed by a suction system designed to prevent turbulence by allowing continuous fluid movement. Temperature was monitored using a thermocouple placed on the slices.

Recordings and electrical stimulation

Extracellular recordings from single neurones in the p.v.n. were obtained using conventional techniques. Glass micropipettes filled with 0·5 M-sodium citrate containing 2 % Pontamine Sky Blue and having a d.c. resistance of 18–35 MΩ were used. Under microscopic observation the electrode was inserted into the p.v.n. which could be distinguished from the surrounding structures visually using transmitted light. Action potentials were displayed on a storage oscilloscope (Tektronix 5000 series) and stored on magnetic tape for further analysis. A window discriminator and an integrator (Nihon Kohden RM 6000 series) were used for continuous observation of the firing patterns of the p.v.n. cells. To quantify the responses following application of peptides, we compared the firing rate in the 5 min before application and the 2 min after application with a 2 min delay to allow for the dead space of perfusion system.

At the end of each recording, the recording site was marked with Pontamine Sky Blue by passing a constant cathodal current through the electrode (1–4 μA for 5 min). Only one mark was made in each slice. After dye-marking the slice was immersed in 4 % paraformaldehyde solution for fixation. The following day, 40 μm-thick frozen sections were cut from the slices with a microtome and mounted on a gelatin-coated glass slide. In order to verify the injection sites of Pontamine Sky Blue dye, the sections were stained with Neutral Red. The recording positions were reconstructed on the maps made on the basis of the distribution of immunohistochemically stained AVP and OXT cells (Swanson & Kuypers, 1980).

To activate the p.v.n. neurones orthodromically, fine bipolar stainless-steel stimulating electrodes were placed dorsal to the columns of the fornix (Dudek, Hatton & MacVicar, 1980). Monophasic single pulses of 20–30 V intensity and 0·1–0·3 ms duration were applied at 1 Hz. Action potentials evoked in p.v.n. neurones following the electrical stimulation were monitored before, during and after application of the low Ca^{2+} and high Mg^{2+} solution.

AVP, OXT and analogues

The peptides used in this experiment were AVP (Peptide Institute, Minoh, Japan), OXT (Peptide Institute, Minoh, Japan), lysine-vasopressin (LVP; Sigma) 1-deamino-8-D-arginine-vasopressin (dDAVP; Ferring), [1-(β-mercapto-, β-cyclopentamethylenepropionic acid)], 8-D-arginine-vasopressin ($d(CH_2)_5$DAVP) and [1-(β-mercapto-, β-cyclopentamethylenepropionic acid), 2-D-tyrosine, 4-valine] arginine-vasopressin ($d(CH_2)_5$D-TyrVAVP). The two AVP analogues, $d(CH_2)_5$DAVP and $d(CH_2)_5$D-TyrVAVP were synthesized in the laboratory of Professor M. Manning, Toledo, OH, U.S.A. (Manning, Lammek, Kruszynski, Seto & Sawyer, 1982*a*; Manning, Olma, Klis & Kolodziejczyk, 1982*b*) and were kindly made available to us by him.

RESULTS

Effects of AVP and OXT

Extracellular recordings were made from a total of ninety-seven spontaneously firing cells in the p.v.n. of the trimmed rat hypothalamic slice preparation. The spontaneously firing cells tested fired at 0·1–8 spikes/s but the majority showed a slow irregular firing pattern. The average firing rate of all ninety-seven cells was $2·2 \pm 0·2$ spikes/s (mean $\pm$ S.E.). Six cells (6%) showed a phasic firing pattern with intermittent bursts of spikes. The amplitude of spikes ranged from 0·5 to 5 mV. Almost all the cells showed stable neural activity during recording and we could record from a single cell for 1–9 h. After taking a stable recording from a single cell for 10 min, peptides were applied to the slices at a concentration of 10^{-7} M. The responses to application of AVP and OXT were assessed by observing the change in firing rate during application of the peptide. The cell was classified as having been excited or inhibited if its firing rate for 2 min after application of the peptide was increased (or decreased) by 20%. Of ninety-seven p.v.n. cells tested, sixty-four (66%) cells showed excitator responses and three (3%) cells inhibitory responses to application of AVP. The average firing rate of the sixty-four cells showing excitatory responses increased from $2·2 \pm 0·3$ to $4·2 \pm 0·4$ spikes/s (mean $\pm$ S.E. of mean) following application of AVP, while that of the three cells showing inhibitory responses decreased from 1·3 to 0·3 spikes/s (mean). Of sixty-eight p.v.n. cells, thirty-nine (57%) cells showed excitatory responses and two (3%) cells inhibitory responses to OXT. The average firing rate of the thirty-nine cells showing excitatory responses increased from $2·2 \pm 0·4$ to $4·3 \pm 0·4$ spikes/s (mean $\pm$ S.E. of mean) following application of OXT, while that of the two cells showing inhibitory responses decreased from 1·7 to 0·7 spikes/s (mean). The results show that there was no practical difference between amounts of change in the firing rate following application of AVP and OXT. Of sixty-six p.v.n. cells, thirty-eight cells responded to both AVP and OXT (excitation, thirty-six cells; inhibition, two cells). Of six phasic firing cells, two showed excitatory responses to AVP and all four cells tested did not show any response to OXT. Examples of excitatory or inhibitory responses of p.v.n. neurones to AVP and OXT are shown in Fig. 1. Most affected cells responded within 2 min and reached maximum or minimum frequency approximately 3 min after perfusion with peptide-containing medium: the sign of the response did not depend on initial firing rate. After washing out the peptides with normal medium, firing rate recovered to pre-treatment values in 5–15 min.

To investigate the effect of peptide concentration, six p.v.n. neurones were tested by applying AVP and OXT at concentrations ranging between 10^{-6} and 10^{-10} M.

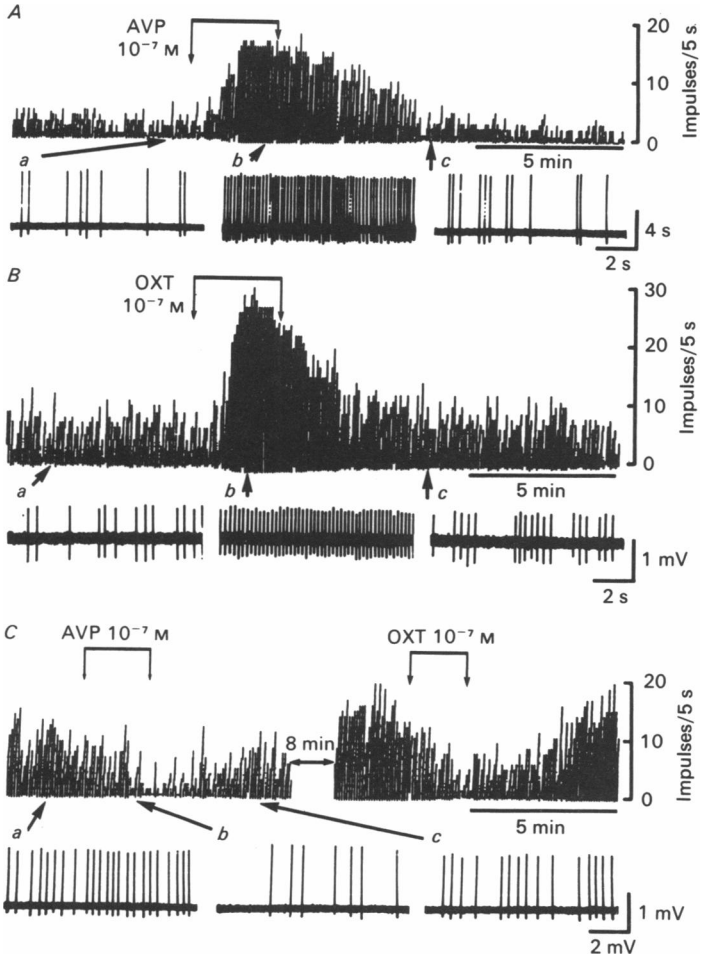

Fig. 1. Responses of p.v.n. neurones to applications of AVP and OXT. *A* and *B* show excitatory responses of p.v.n. cells following application of AVP 10^{-7} M and OXT 10^{-7} M respectively (indicated by bars). *C* shows inhibitory responses of a single p.v.n. cell following application of AVP 10^{-7} M and OXT 10^{-7} M respectively. The upper trace in each illustration shows a rate-meter record and the lower trace oscilloscope sweeps before (*a*), during (*b*) and after (*c*) application of the peptides.

Fig. 2*A* shows a representative example of the effects of AVP on a p.v.n. neurone. From the rate-meter records, the increase from the resting level following application of the peptide at each concentration was calculated and plotted against the concentration (Fig. 2*B*). A similar analysis, performed on another p.v.n. cell after application of OXT is shown in Fig. 2*C*. Both showed a direct relationship between the increase in firing rate and the concentration of the peptide. The threshold concentration of AVP and OXT applied to the p.v.n. cells tested ranged from 10^{-8} to 10^{-10} M. The excitatory effects of AVP and OXT were reversible and tachyphylaxis was not observed with repeated application of the peptides (see Fig. 7). The results

indicated that most of the responsive p.v.n. cells showed excitatory responses to application of the peptides. However, we observed inhibitory responses in a few p.v.n. cells both to AVP and OXT.

Distribution of peptide-responsive neurones in the p.v.n.

We investigated the distribution of peptide-responsive neurones within the p.v.n. Fig. 3 shows the recording sites in the p.v.n. histologically determined by means of Pontamine Sky Blue marking. The shaded regions indicate the areas in the p.v.n.

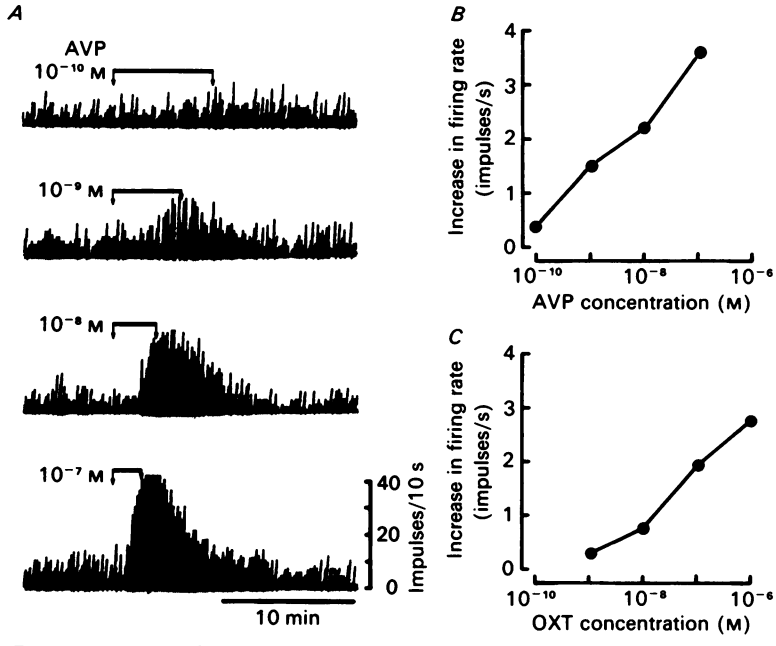

Fig. 2. Dose–responses of p.v.n. neurones to AVP and OXT. *A* shows rate-meter records of the responses of a p.v.n. cell to different concentrations of AVP (10^{-10}–10^{-7} M). The threshold concentration to evoke the responses of the cell was 10^{-10} M for AVP. Note that the response to AVP 10^{-7} M is out of range. *B* shows a dose–response curve for AVP obtained from the p.v.n. cell shown in *A*. *C* shows a dose–response curve for OXT obtained from another p.v.n. cell.

where cell bodies stained immunocytochemically for AVP and OXT (Swanson & Kuypers, 1980). The location of eighty-three of the ninety-seven p.v.n. cells tested was reconstructed from the Pontamine Sky Blue marks. The results did not show any grouping of the responsive cells inside the p.v.n. They were diffusely distributed throughout the nucleus.

Effects of peptides in the absence of synaptic transmission

A low Ca^{2+} and high Mg^{2+} medium was used to block synaptic transmission. Action potentials evoked in p.v.n. cells following electrical stimulation of the area dorsal to the fornix in normal perfusion medium (Fig. 4*A*) disappeared after 1·7–8·8 min (4·0 ± 0·5 min, mean ± S.E. of mean, $n = 12$) in the low Ca^{2+} and high Mg^{2+} perfusion

medium (Fig. 4*B*). Recovery of synaptic transmission after washing out the low Ca^{2+} and high Mg^{2+} medium with normal perfusion medium took 2·5–11·5 min (4·9 ± 0·8 min, mean ± S.E. of mean, $n = 12$) (Fig. 4*D*). These results suggest that all synaptic transmission was blocked when the slices were perfused with the low Ca^{2+} and high Mg^{2+} medium for more than 15 min and that under these conditions the

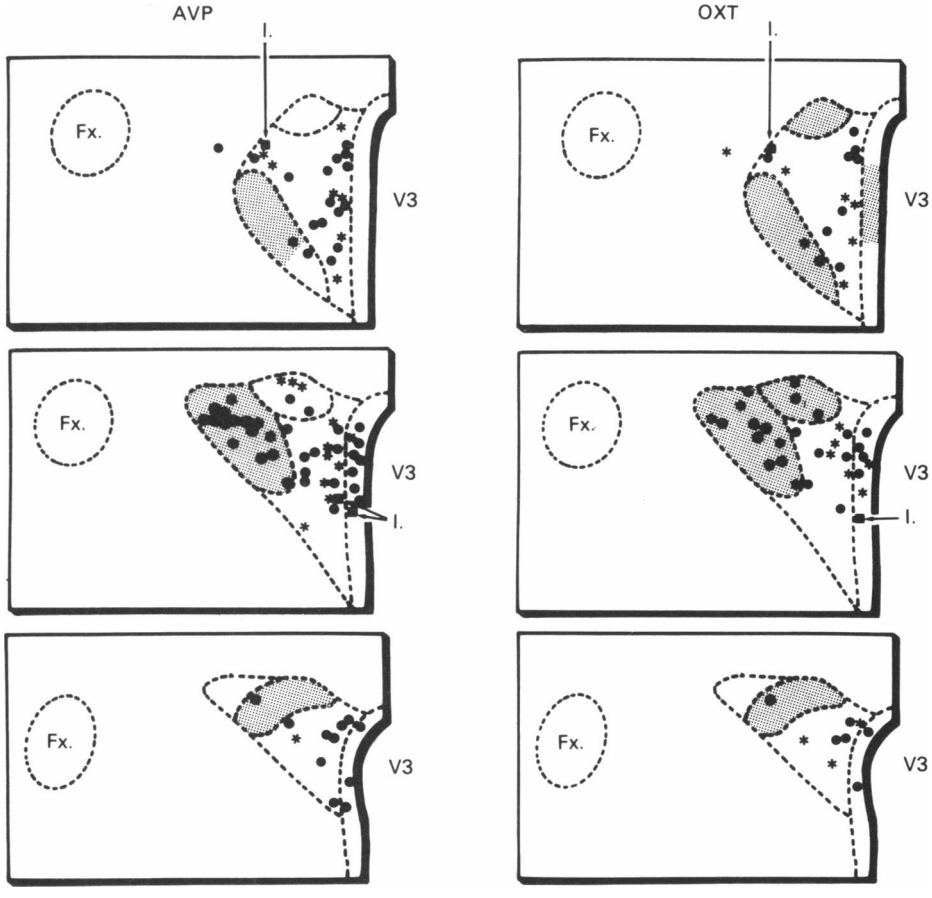

Fig. 3. Distribution of recording sites of p.v.n. cells. Each illustration shows a schematic map of coronal section of the trimmed hypothalamus made by immunocytochemical staining. Shaded areas in the maps indicate principal sites of cell bodies of AVP (left) and OXT (right) containing cells. Circles indicate location of cells which showed excitatory responses, squares inhibitory responses (indicated by 'I.') and stars no response, respectively. The maps from the top to the bottom represent the frontal sections from rostral to caudal. Abbreviations: Fx., fornix; V_3, third ventricle.

spontaneous spikes of cells were due largely to their own intrinsic activity. In general, application of the low Ca^{2+} and high Mg^{2+} medium decreased the activity of p.v.n. cells. However, a few p.v.n. cells showed an increased spontaneous discharge rate under these conditions.

After blocking synaptic transmission with such procedures, responses of p.v.n. cells

to AVP and OXT were investigated. Application of AVP increased the firing rate of fifteen p.v.n. cells (from $0{\cdot}9 \pm 0{\cdot}4$ to $2{\cdot}8 \pm 0{\cdot}6$ spikes/s, mean ± S.E. of mean), which had shown excitatory responses to AVP in normal medium (from $2{\cdot}1 \pm 0{\cdot}6$ to $4{\cdot}3 \pm 0{\cdot}7$ spikes/s, mean ± S.E. of mean). Fourteen cells which increased their firing rate by OXT in normal medium (from $2{\cdot}0 \pm 0{\cdot}6$ to $4{\cdot}7 \pm 0{\cdot}8$ spikes/s, mean ± S.E. of mean) were

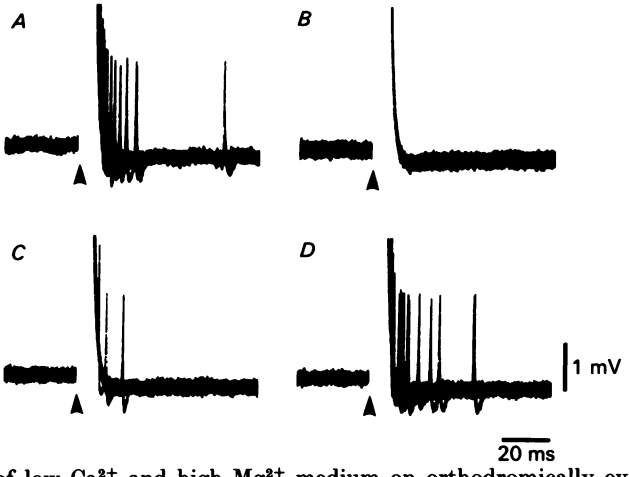

Fig. 4. Effects of low Ca^{2+} and high Mg^{2+} medium on orthodromically evoked action potentials of p.v.n. cells. *A* shows evoked action potentials following stimulation (indicated by arrowheads, 20 V, 0·3 ms, 1 Hz) of the area dorsal to the fornix. As shown in *B*, no action potential was evoked 150 s after perfusing with a low Ca^{2+} and high Mg^{2+} medium. *C* and *D* show the recovery process in 3 min and 8 min respectively, after perfusing with a normal medium. Each recording shows twenty superimposed oscilloscope tracings.

also excited by OXT after blockade of synaptic transmission (from $1{\cdot}1 \pm 0{\cdot}4$ to $2{\cdot}9 \pm 0{\cdot}7$ spikes/s, mean ± S.E. of mean). Fig. 5 shows examples of the excitatory responses of two p.v.n. cells to AVP and OXT after blocking synaptic transmission; both cells had shown excitatory responses to both peptides in normal medium. The magnitude of the responses of the cells to AVP and OXT after blocking synaptic transmission was often smaller than it was before blockade. Such a tendency could be found in half the cells tested. Two cells which had responded to both AVP and OXT in normal medium responded to one of the peptides with the same magnitude but responded to the other peptide with a reduced magnitude after blocking synaptic transmission (Fig. 5*C* and *D*). These results suggest that some p.v.n. cells are themselves sensitive to AVP and OXT and also may receive some synaptic inputs from sensitive cells located in or near the p.v.n., while we cannot exclude the possibility that medium with low Ca^{2+} and high Mg^{2+} exerts a depressive effect by changing the membrane characteristics.

To investigate the dose–responses of p.v.n. cells to the peptides in the absence of synaptic transmission, different concentrations of AVP and OXT were applied to p.v.n. cells before and after blockade of synaptic transmission. After blockade of synaptic transmission, the cells continued to respond to both AVP and OXT in a dose-dependent manner without major changes in the threshold or responsiveness. This indicates that the p.v.n. cells themselves show dose-dependent excitatory

responses to AVP and OXT. Since inhibitory responses were encountered so rarely, it was not possible to test whether they also remained after synaptic blockade.

AVP receptors

Two kinds of AVP receptors are known, i.e. V_1 and V_2 receptors. The V_1 or vasopressor receptor present on vascular smooth muscle cells and on liver cells triggers the vasopressor and glycogenolytic effects of vasopressin in these tissues

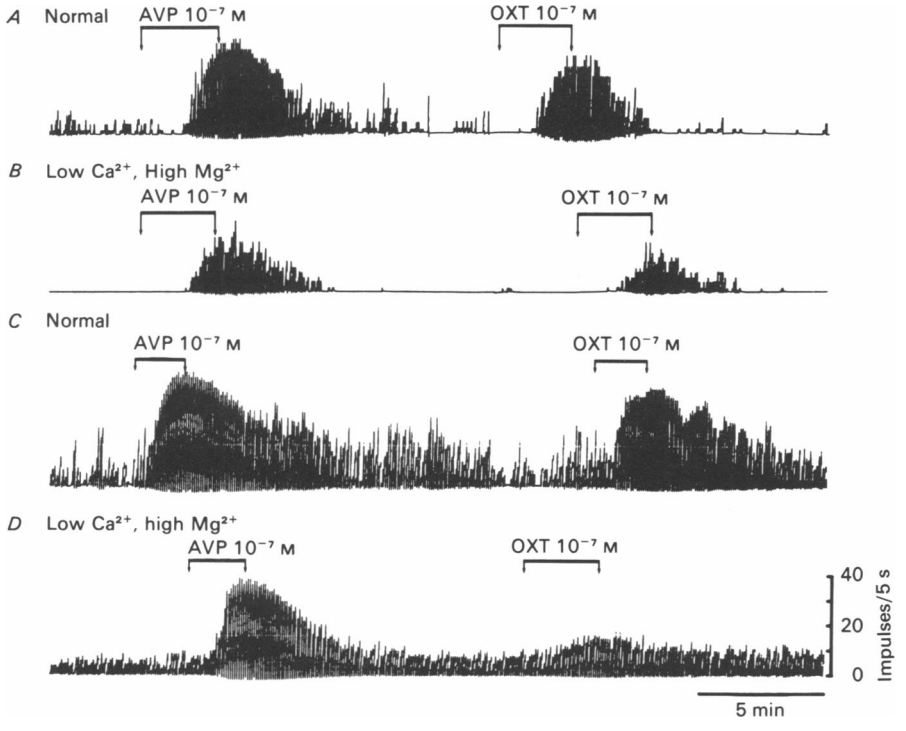

Fig. 5. Rate-meter records showing the effects of blockade or synaptic transmission on the response of p.v.n. cells to application of AVP and OXT. *A* shows responses of a p.v.n. cell to application of AVP 10^{-7} M and OXT 10^{-7} M in a normal medium. *B* shows the responses of the same cell to AVP and OXT after perfusing with a low Ca^{2+} and high Mg^{2+} medium. Note the decreased response in the low Ca^{2+} and high Mg^{2+} medium. *C* and *D* show responses taken from another cell to AVP and OXT in the normal and the low Ca^{2+} and high Mg^{2+} medium respectively. This cell responded both to AVP and OXT after blockade of synaptic transmission. However, the magnitude of the responses to OXT was strongly suppressed in the low Ca^{2+} and high Mg^{2+} medium.

(Jard, 1981), while the V_2 or antidiuretic receptor is related to activation of adenylate cyclase by vasopressin in renal tubular cells (Butlen, Guillon, Rajerison, Jard, Sawyer & Manning, 1978). We investigated which type of receptor was involved in the responses of p.v.n. cells to AVP, using AVP analogues. The AVP analogues used in the experiment were $d(CH_2)_5$DAVP as V_1-receptor antagonist, $d(CH_2)_5$D-TyrVAVP as V_1/V_2-receptor antagonist and dDAVP as V_2-receptor agonist. Analogue $d(CH_2)_5$DAVP is one of the most specific vasopressor antagonists available and

$d(CH_2)_5$D-TyrVAVP has moderate antagonistic effects on V_2 receptors as well as strong antagonistic effects on V_1 receptors (Manning *et al.* 1982*a*, *b*). Fig. 6 shows the effects of the V_1-receptor antagonist and the V_2-receptor agonist on the spontaneous and AVP-induced activities of a p.v.n. cell. The cell was excited by application of AVP 10^{-7} M (Fig. 6*A*), while application of the V_2-receptor agonist did not lead to

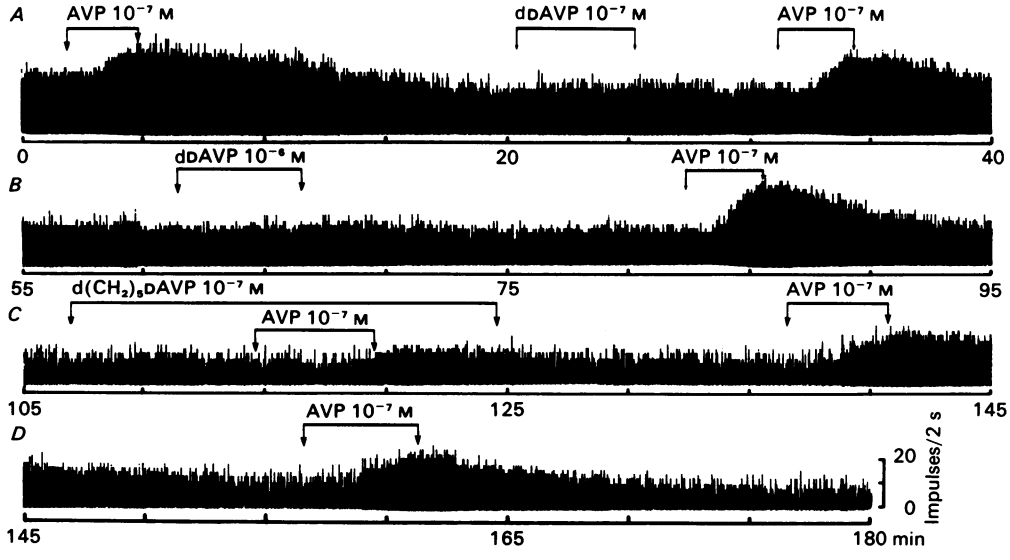

Fig. 6. Rate-meter records to show the effects of V_1 antagonist and V_2 agonist on the spontaneous and AVP-induced activities of a p.v.n. cell. Application of AVP 10^{-7} M increased the firing rate but that of V_2 agonist (dDAVP 10^{-7} M and 10^{-6} M) did not change it. Application of V_1 antagonist ($d(CH_2)_5$DAVP 10^{-7} M) completely suppressed the activities induced by AVP 10^{-7} M, while that of V_1 antagonist itself did not change the activity. Time from the start of recording is indicated under the trace.

any increase in the firing rate even at a concentration of 10^{-6} M (Fig. 6*B*). On the other hand, the V_1-receptor antagonist completely blocked the effects of AVP on the p.v.n. cell while the V_1-receptor antagonist did not alter the spontaneous discharge of the cell. After application of the V_1-receptor antagonist, the AVP-induced responses were reduced for more than 60 min.

Fig. 7 shows the effects of the V_1-receptor antagonist, $d(CH_2)_5$DAVP, and of the V_1/V_2-receptor antagonist, $d(CH_2)_5$D-TyrVAVP on AVP- and OXT-induced responses. The V_1/V_2-receptor antagonist partly supressed the effects of AVP while the V_1-receptor antagonist completely blocked its effect. Of twelve p.v.n. cells tested, $d(CH_2)_5$DAVP ($n = 10$) and $d(CH_2)_5$D-TyrVAVP ($n = 3$) 10^{-7} M completely or partly blocked the effects of AVP (10^{-8} and 10^{-7} M) on the p.v.n. neurones. These antagonists, $d(CH_2)_5$DAVP ($n = 2$) and $d(CH_2)_5$D-TyrVAVP ($n = 1$) 10^{-7} M also partly blocked the effects of OXT (10^{-7} M) on the p.v.n. neurones. On the other hand, all of the six p.v.n. cells showing an excitatory response to application of AVP 10^{-7} M were unaffected by application of dDAVP 10^{-7} and 10^{-6} M. Thus, from the results obtained with the AVP analogues, it may be concluded that p.v.n. cells which responded to AVP have V_1-type receptors.

Different mode of responses on single p.v.n. neurones by application of AVP and OXT

The magnitude of the responses of p.v.n. cells to application of AVP and OXT varied from cell to cell. Thus, we systematically analysed the responses to AVP and OXT of sixty-six p.v.n. cells, where both peptides were applied successfully at 10^{-7} M. According to their responses, the cells could be divided into four almost equal groups

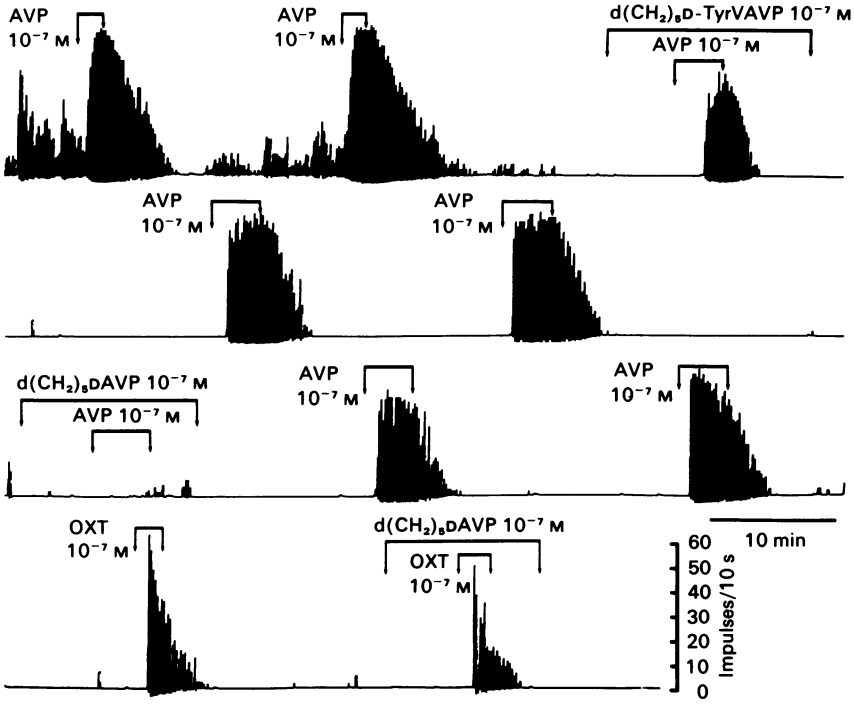

Fig. 7. Effect of V_1 antagonist and V_1/V_2 antagonist on the AVP- and OXT-induced activities of a p.v.n. neurone. Application of AVP 10^{-7} M excited the cell. Tachyphylaxis was not observed with repeated application of AVP. The V_1 antagonist, $d(CH_2)_5$DAVP, completely blocked the effect of AVP on this cell but the V_1/V_2 antagonist, $d(CH_2)_5$D-TyrVAVP, only partly suppressed the effect of AVP. Application of OXT 10^{-7} M also excited the cell. The V_1 antagonist, $d(CH_2)_5$DAVP, however, partly blocked the effect of OXT on the cell. The four charts are continuous rate-meter recordings of the p.v.n. neurone.

as follows. (i) Cells which showed responses to AVP greater than that to OXT (twenty cells) (see Fig. 8*A*). (ii) Cells which showed responses to AVP which were very similar to one of OXT (sixteen cells) (see Fig. 8*B*). (iii) Cells which showed responses to AVP which were smaller than that to OXT (fifteen cells) (see Fig. 8*C*). (iv) Cells which did not respond to either peptide (fifteen cells). Cells which responded to only one of the peptides were included in group (i) or (iii) (fourteen cells). The results indicate that the responses to each peptide are different from cell to cell and that a cell may have receptors both for AVP and OXT but in different proportions.

Effects of LVP

We also investigated the effects of LVP on six p.v.n. neurones which showed excitatory responses to AVP and on one p.v.n. cell which showed inhibitory responses to AVP. As shown in Fig. 8*A*, all tested cells responded to LVP in the same way as to AVP but their responses to LVP were always smaller. One p.v.n. cell did not

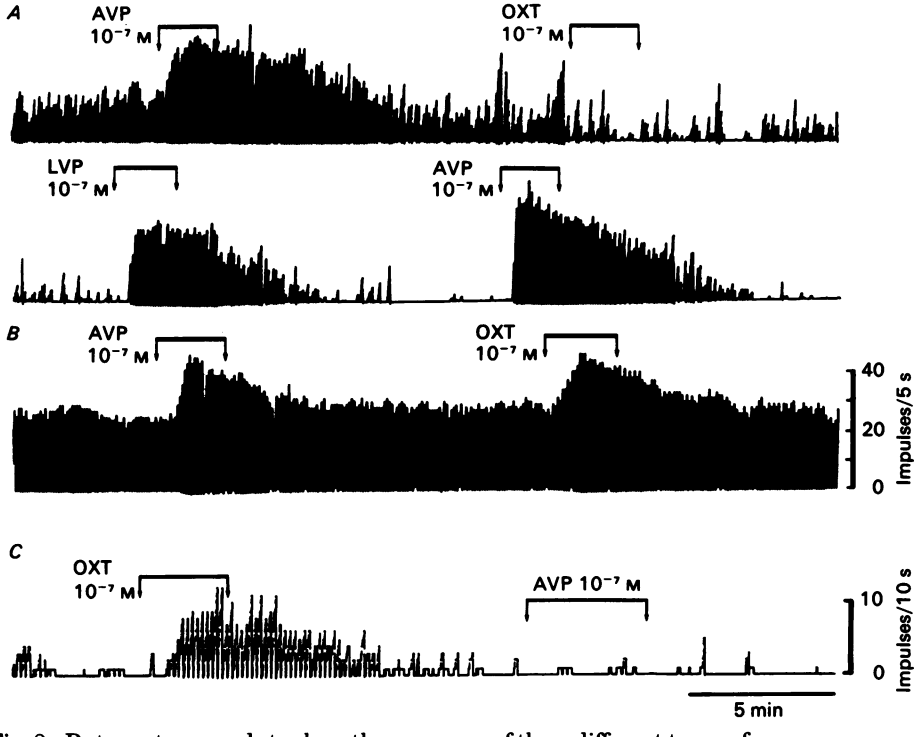

Fig. 8. Rate-meter records to show the responses of three different types of p.v.n. neurones to application of AVP and OXT. The responses of p.v.n. cells following application of AVP and OXT varied from cell to cell. In *A*, the responses to AVP were bigger than that to OXT. In *B*, the response to AVP was equal to that to OXT. In *C*, the response to AVP was smaller than that to OXT. In *A*, the response of p.v.n. cells to LVP was also shown.

respond to OXT but responded to AVP and LVP. The LVP-induced responses were reversible and reproducible with repeated application of LVP. Thus LVP like AVP probably affects p.v.n. cells through V_1 receptors.

Effects of AVP and OXT on cells in other than the p.v.n.

To test whether the AVP and OXT used in the present experiments had non-selective effects on all hypothalamic neurones, AVP and OXT were applied to an identifiable group of hypothalamic neurones outside the p.v.n. A total of twenty cells in the s.c.n. were tested. Following application of AVP, four cells out of twenty showed a weak excitation (20–30 % increase in the firing rate) at 10^{-7} M and OXT also produced a weak excitatory response at 10^{-7} M on four out of nineteen cells. The analysis showed

that the number of peptide-responsive p.v.n. cells (excitation and inhibition) was significantly greater than that of s.c.n. cells (AVP: $\chi^2 = 8{\cdot}32$, $P < 0{\cdot}004$; OXT: $\chi^2 = 9{\cdot}17$, $P < 0{\cdot}002$ at d.f. = 1).

DISCUSSION

The concentration of the peptides used in these experiments ranged from 10^{-10} to 10^{-6} M and the threshold concentration was approximately 10^{-10} M. However, normal concentrations of AVP and OXT in the cerebrospinal fluid (c.s.f.) of rats are known to be 10^{-11} and 7×10^{-11} M, respectively (Dogterom, Van Wimersma Greidanus & Swaab, 1977). The question thus arises whether the effects reported here with a 10–100-fold greater concentration of AVP and OXT are of physiological significance. There are many possible explanations for this difference. If AVP and OXT were released locally upon p.v.n. neurones and acted as neurotransmitters or neuromodulators, the concentration of the peptides near the cell body might be much higher than that in the c.s.f. Therefore, it seems reasonable to consider that the concentration range used in this study might be in the physiological range. In addition, the threshold concentration of the peptides used in our experiments was similar to that applied by other workers to rat motoneurones (Suzue *et al.* 1981) and rat hippocampal neurones (Mühlethaler *et al.* 1982, 1983).

In our experiments, p.v.n. neurones responded to AVP and OXT with a relatively short latency and recovered rapidly after washing out of peptides with normal medium. Tachyphylaxis was not observed with repeated applications of the peptides. The responses closely resembled those obtained from rat hippocampal neurones following application of AVP and OXT (Mühlethaler *et al.* 1982, 1983). Rapid responses to and rapid recovery from application of LVP to s.o.n. neurones of guinea-pigs in slice preparations have also been observed (Abe *et al.* 1983).

AVP is known to act as a vasoconstrictor affecting vascular smooth muscle of the vasculature through V_1 receptors and to exert an antidiuretic effect through V_2 receptors in the kidney. We therefore tried to answer which type of receptors were involved in AVP-induced responses of the p.v.n. by using AVP analogues as antagonists. V_1- or V_1/V_2-receptor antagonists completely or partly blocked AVP-induced excitatory responses of p.v.n. neurones, while a powerful V_2-receptor agonist, dDAVP, did not affect the spontaneous discharge of the neurones. The results strongly suggest that AVP-induced responses of the p.v.n. cells may be mediated through V_1-type receptors. However, application of LVP to the s.o.n. cells of guinea-pigs induced activation of adenylate cyclase which was closely coupled to the V_2-type receptor and inhibited the firing rate (Abe *et al.* 1983).

Additional evidence for inhibitory effects of AVP on phasic firing s.o.n. cells was reported *in vitro* by Leng & Mason (1982). However, some phasic firing p.v.n. cells were excited but none of the cells were inhibited by AVP in the present experiment. We observed only three non-phasic firing cells which showed inhibitory responses to AVP and only two to OXT. It is believed that magnocellular neurones fire only slowly or become silent after denervation (Dyball & Dyer, 1971) or in slice preparations (Haller & Wakerley, 1980) and it is known that the magnocellular parts of the p.v.n. also contain parvocellular cells (Swanson & Kuypers, 1980). Therefore, it is possible

that most of the p.v.n. cells recorded in the present study were parvocellular neurones. The cells which showed inhibitory responses might therefore have been magnocellular cells. It is thus possible that AVP exerts excitatory effects through V_1-type receptors and inhibitory effects through V_2-type receptors on different populations of neurones. Several previous studies have reported that there was a difference in the sensitivity of neurones in the different parts of the C.N.S. to application of AVP and OXT. For example rat hippocampal neurones responded selectively to OXT at a tenth of the concentration required for AVP (Mühlethaler *et al.* 1983) and the s.o.n. cells of guinea-pigs responded selectively to AVP at one-hundredth of the concentration required for OXT (Abe *et al.* 1983). We could not however, observe such selective responses in the p.v.n. cells. If the response of p.v.n. neurones to AVP and OXT were mediated by the same type of receptor, the ratio of the responses of each p.v.n. neurone to each peptide should be uniform. Since this was not the case, it may be concluded that there are several types of cells present in the p.v.n. which had both AVP and OXT receptors in different proportions. The situation in the p.v.n. is thus unlike that in the s.o.n. or the hippocampus where the affinity for one of the peptides was stronger than for the other.

The specificity of the AVP, OXT and analogues used in this experiment needs some consideration. The analogues of AVP used in this experiment were not specific only for AVP receptors but also affected OXT receptors (Manning & Sawyer, 1984). At present, there is no specific analogue for AVP receptors. Highly selective analogues for OXT receptors exist (Mühlethaler *et al.* 1983), but were not available for use in our experiment. A further study using specific analogues for OXT receptors could give a more complete picture of the receptors in the p.v.n. Non-selective effects of the peptides used in the experiments also need to be excluded by testing a population of hypothalamic neurones outside the p.v.n. The responses of the cells in the s.c.n. to the peptides were weaker and significantly less frequent than those in the p.v.n. Further, AVP-induced responses could be completely blocked by a vasopressin analogue. Thus it is highly unlikely that the responses we observed were non-specific effects of AVP and OXT. It is much more likely that the high sensitivity of p.v.n. cells to the peptides is physiologically meaningful.

It is also of interest to know whether the responses to AVP and OXT are different in the different subdivisions of the p.v.n., whether for example certain cells in a particular site can respond to the peptides. Immunocytochemical studies have shown that most of the cell bodies of AVP- and OXT-containing cells are situated in the magnocellular parts of the p.v.n. (e.g. Swanson & Kuypers, 1980). On the other hand, there are many parvocellular cells present in the p.v.n., which do not contain AVP and OXT. As shown in Fig. 3, AVP- and OXT-responsive neurones were not located at special sites but were distributed throughout the p.v.n. However, we recorded more spontaneously active cells in the parvocellular parts of the p.v.n. than in the magnocellular parts. Further, the spontaneous activity of neurosecretory cells in the s.o.n. of rat slice preparations is very low (Haller & Wakerley, 1980). Since in slice preparations it is impossible to use antidromic stimulation to identify magnocellular neurosecretory cells, we feel that in our slice preparations, most of the p.v.n. cells recorded in the present study may not have been magnocellular neurosecretory cells but parvocellular cells.

Swanson & Sawchenko (1980) suggested that the p.v.n. played a role in the in-

tegration of neuroendocrine and autonomic functions and there are direct connexions from parvocellular cells in the p.v.n. to the medulla. We could imagine that locally released AVP and OXT from magnocellular neurosecretory cells in the p.v.n. by an unknown mechanism directly affected parvocellular cells in the p.v.n. and probably through peptide-sensitive cells in or near the p.v.n. These intranuclear connexions may contribute to the integration of neuroendocrine and autonomic functions. Another possible function of intranuclear connexions mediated by released AVP and OXT may be an autoregulation, involving recurrent inhibition or facilitation of the neurosecretory cells (Koizumi & Yamashita, 1971; Koizumi, Ishikawa & Brooks, 1973; Akaishi & Ellendorff, 1983). Neurosecretory cells might therefore be affected both directly and indirectly by the peptides.

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