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EFFECTS OF ATRIAL NATRIURETIC POLYPEPTIDE ON RAT HYPOTHALAMIC NEURONES *IN VITRO*

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SUMMARY

1. Extracellular recordings were made from 175 spontaneously active cells in the rat coronal hypothalamic slice preparation. Reconstruction of the recording sites showed that fifteen were in the supraoptic nucleus (s.o.n.), ten in the magnocellular portion of the paraventricular nucleus (p.v.n.) which could be antidromically activated by stimulation lateral to the nucleus, seventy-seven other cells in the p.v.n. and seventy-three in the anteroventral third ventricle (a.v.3.v.) region.

2. The mean firing rates (mean ± S.E. of mean) of the spontaneously firing cells in the s.o.n., p.v.n. and a.v.3.v. were 2·8 ± 0·4 spikes/s, 2·9 ± 0·2 spikes/s and 5·0 ± 0·4 spikes/s, respectively. Antidromically identified p.v.n. cells fired spontaneously with a mean firing rate of 1·5 ± 0·5 spikes/s.

3. Bath application of atrial natriuretic polypeptide (a.n.p.; 10^{-7} M) had no effect on fifteen s.o.n. cells tested but nineteen (22%) of eighty-seven p.v.n. cells (including two of the ten antidromically activated cells) and thirty (41%) of seventy-three a.v.3.v. cells showed inhibitory responses. Three (3%) cells in the p.v.n. were excited by a.n.p.

4. The dose dependence of the response to a.n.p. was tested in two p.v.n. and five a.v.3.v. cells. As a.n.p. concentration increased, the firing rates of all seven cells generally decreased. However, one a.v.3.v. neurone was excited at low concentrations (less than 10^{-8} M) but inhibited at high concentrations (10^{-7} and 10^{-6} M) of a.n.p. The threshold concentration to evoke inhibitory responses in the p.v.n. was 10^{-10} M and in the a.v.3.v. was 10^{-11} M.

5. With the exception of the two antidromically activated p.v.n. cells, the inhibitory effect of a.n.p. still persisted after synaptic transmission had been suppressed with a low-Ca^{2+} and high-Mg^{2+} medium.

6. Thirty-six cells in the a.v.3.v. were tested with both a.n.p. and angiotensin II applied at 10^{-7} M. Twelve showed inhibitory responses to a.n.p. and nine showed excitatory responses to angiotensin II. In other experiments, a.n.p., angiotensin II and arginine-vasopressin were each applied to neurones in the p.v.n. Of the forty cells tested with all three peptides at 10^{-7} M, seven were inhibited by a.n.p., fourteen were

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excited by angiotensin II and twenty were excited by arginine-vasopressin. No neurones in either the p.v.n. or a.v.3.v. were inhibited by a.n.p. and excited by angiotensin II, but four neurones in the p.v.n. were inhibited by a.n.p. and excited by arginine-vasopressin.

7. We conclude that a.n.p. has central actions which include a direct inhibitory effect on neurones in the a.v.3.v. and parvocellular p.v.n. but no direct effect on magnocellular neurones in either the s.o.n. or p.v.n. and that a.n.p. may have an opposite action to angiotensin II and arginine-vasopressin in both the p.v.n. and a.v.3.v.

INTRODUCTION

It has been known for over 20 years that some muscle cells in mammalian cardiac atria contain secretory granules, but the function of these granules remained obscure. More recently, a peptide, called atrial natriuretic polypeptide (a.n.p.), isolated from the atria has been shown to have a powerful natriuretic action and a relaxant effect on smooth muscle (Flynn, de Bold & de Bold, 1983; Currie, Geller, Cole, Siegel, Fok, Adams, Eubanks, Galluppi & Needleman, 1984). Thus the renal and vascular actions of a.n.p. appear to be opposite to those of arginine-vasopressin, which has antidiuretic and vasoconstrictive effects.

It has also been demonstrated that immunoreactive a.n.p. can be extracted from the rat hypothalamus as well as both atria and plasma (Tanaka, Misono, & Inagami, 1984) and several immunohistochemical studies have demonstrated that a.n.p. immunoreactivity exists in the central nervous system, especially in the hypothalamus (Saper, Standaert, Currie, Schwartz, Geller & Needleman, 1985; Jacobowitz, Skofitsch, Keiser, Eskay & Zamir, 1985; Kawata, Nakao, Morii, Kiso, Yamashita, Imura & Sano, 1985). Other studies have shown that a.n.p. inhibits angiotensin II-induced vasoconstriction in the aorta (Kleinert, Maack, Atlas, Januszewicz, Sealey & Laragh, 1984), angiotensin II-induced drinking (Nakamura, Katsuura, Nakao & Imura, 1985; Antunes-Rodrigues, McCann, Rogers & Samson, 1985), and dehydration- and haemorrhage-induced arginine-vasopressin release (Samson, 1985). These observations suggest that a.n.p. interacts with angiotensin II and/or arginine-vasopressin in the central nervous system, but its physiological significance there is still incompletely understood.

We conducted the present experiments using the rat brain-slice preparation to determine whether a.n.p. had any direct effects on single cells in the supraoptic nucleus (s.o.n.), paraventricular nucleus (p.v.n.) and anteroventral third ventricle region (a.v.3.v.) all of which are directly involved in the control of arginine-vasopressin release.

METHODS

Adult male Wistar rats weighing 150–300 g were stunned and decapitated. The brains were then quickly removed and blocked with a razor blade. Coronal brain slices 350–450 μm in thickness containing the s.o.n., p.v.n. and a.v.3.v. were cut with a vibratome-type slicer. Five or six slices of hypothalamic tissue were usually obtained from each brain. Immediately after sectioning the slices were placed in incubation medium oxygenated with a mixture of 95% O_2 and 5% CO_2 at

room temperature and left for at least 1 h until they were transferred to a recording chamber. The incubation medium was a modified Yamamoto's solution (pH 7·3–7·5), which contained (in mM): NaCl, 124; KCl, 5; KH_2PO_4, 1·24; $MgSO_4$, 1·3; $CaCl_2$, 2·1; $NaHCO_3$, 20; and glucose, 10 (Inenaga & Yamashita, 1986; Okuya, Inenaga, Kaneko & Yamashita, 1987). In the perfusing medium, Ca^{2+} concentration was always lowered to 0·75 mM to increase spontaneous activity of s.o.n. and p.v.n. magnocellular neurones (Pittman, Hatton & Bloom, 1981; Okuya *et al.* 1987). When required, a low-Ca^{2+} (0·5 mM) and high-Mg^{2+} (9 mM) solution was used in an attempt to block synaptic transmission (Inenaga & Yamashita, 1986). The pH of the perfusing medium was not affected by adding peptides at the concentrations used.

Before the experiments started, each slice was carefully trimmed with a microsurgery knife so that recordings were made from a piece of tissue containing the s.o.n. or p.v.n. or a.v.3.v. area with a total area of less than 2 × 2 mm. The trimmed slice containing the s.o.n. also contained a part of the perinuclear zone of the s.o.n. and a part of the optic chiasm (Fig. 1*A*). The trimmed slice of the p.v.n. also contained a part of the anterior hypothalamus, zona incerta and fornix (Fig. 1*B*). The preparation of the a.v.3.v. mainly contained the organum vasculosum of the lamina terminalis, preoptic suprachiasmatic nucleus, periventricular preoptic nucleus and the median preoptic nucleus (Fig. 1*C*). Larger slices with the full perifornical zone were used when the axons of the p.v.n. magnocellular cells were to be stimulated antidromically.

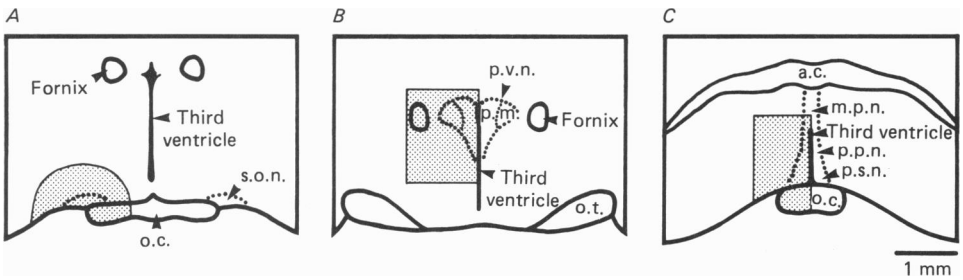

Fig. 1. Diagrams of the hypothalamic slice preparations. *A*, *B* and *C* show representative slices containing the supraoptic nucleus (s.o.n.), paraventricular nucleus (p.v.n.) and anteroventral third ventricle (a.v.3.v.) regions, respectively. Shaded regions show the parts trimmed from the coronally cut slices. Abbreviations: a.c., anterior commissure; m., magnocellular portion; m.p.n., median preoptic nucleus; o.c., optic chiasm; o.t., optic tract; p., parvocellular portion; p.p.n., periventricular preoptic nucleus; p.s.n., preoptic suprachiasmatic nucleus.

The trimmed slice was placed on a Sylgard mat glued to the bottom of the recording chamber which had a volume of 0·8 ml and was held in place with a nylon net and platinum weights. The temperature of the perfusing medium which was oxygenated with a mixture of 95% O_2 and 5% CO_2 was kept at 35 ± 0·5 °C. The perfusion system was gravity-fed and the flow rate of perfusing medium in the recording chamber was adjusted to 1–2 ml/min. The perfusing medium in the chamber could be completely exchanged in 1 min. The all-perfusion system was completely siliconized. Peptides were applied to the slice by perfusing from separate storage bottles containing medium to which they had been added.

Extracellular recordings from single neurones in the s.o.n., p.v.n. and a.v.3.v. were obtained using conventional techniques. Glass micropipettes filled with 0·5 M-sodium acetate containing 2% Pontamine Sky Blue and having a d.c. resistance of 20–35 MΩ were used. The electrodes were directed into the relevant regions of the slices which could be readily distinguished from the surrounding structures visually using a microscope and transmitted light. Action potentials were recorded using a conventional amplifier with a frequency range of d.c.-30 Hz, displayed on a storage oscilloscope and stored on magnetic tape for further analysis. A window discriminator and an integrator were used for continuous observation of the firing patterns of cells in each region. At the end of recording, a constant cathodal current of 5 μA was passed through the tip of the electrode for 3–5 min to deposit a blue spot from which the recording sites could be precisely determined histologically.

In order to activate the p.v.n. magnocellular neurones antidromically, fine bipolar stimulating

electrodes, which were made from two stainless-steel wires and insulated except at the tips (diameter: 100 μm; separation: 0·5 mm), were placed dorsolateral to the column of the fornix along the course of the axons of p.v.n. magnocellular neurones projecting to the neural lobe (Krieg, 1932; Dudek, Hatton & Macvicar, 1980). Monophasic single pulses of 200 μs duration and less than 0·8 mA intensity were delivered at 1 Hz.

The responses were assessed by observing the change in firing rate before and during application of peptides. The change in firing rate from the resting level (mean firing rate during 5 min) to the peak or trough level (mean firing rate during 1 min) following application of peptides was calculated from the rate-meter records. Cells were classified as having been excited or inhibited if their firing rates after application of peptides were increased or decreased by more than 20%.

The peptides used in the experiments were atrial natriuretic polypeptide (a.n.p.; Rat 1–28, 4151-v Lot No. 341216, Peptide Institute, Minoh, Japan), angiotensin II and arginine-vasopressin (both from the Peptide Institute, Minoh, Japan). The biological activities of the synthetic a.n.p. were determined using bioassay preparations. The purity of the peptide was checked by a specific optical rotation, elemental analysis, amino acid analysis, thin layer chromatography and high performance liquid chromatography. The total amount of impurities detected was less than 1%.

RESULTS

Effects of atrial natriuretic polypeptide on supraoptic nucleus, paraventricular nucleus and anteroventral third ventricle neurones

Extracellular recordings were made from fifteen spontaneously firing cells in the s.o.n., seventy-seven spontaneously firing cells in the p.v.n. and seventy-three spontaneously firing cells in the a.v.3.v. The s.o.n. neurones generally showed a slow and irregular firing pattern, while most of the p.v.n. and a.v.3.v. neurones showed a continuous or irregular firing pattern. The mean firing rate (mean $\pm$ S.E. of mean) of the spontaneously firing cells in the s.o.n., p.v.n. and a.v.3.v. was $2{\cdot}8 \pm 0{\cdot}4$ spikes/s, $3{\cdot}1 \pm 0{\cdot}2$ spikes/s and $5{\cdot}0 \pm 0{\cdot}4$ spikes/s, respectively. The amplitude of spikes recorded in this study ranged from 0·6 to 5·0 mV.

After making a stable recording from a single cell for at least 10 min, a.n.p. was applied to the slice at a concentration of 10^{-7} M. None of the fifteen s.o.n. cells tested were inhibited but seventeen (22%) of seventy-seven p.v.n. cells and thirty (41%) of seventy-three a.v.3.v. cells showed inhibitory responses to application of a.n.p. (Fig. 2*A*, *B*, *C* and *D*). Only three (4%) of the p.v.n. cells showed excitatory responses to a.n.p. (Fig. 2*E*). The proportion of cells inhibited by a.n.p. was significantly higher in the a.v.3.v. than in the s.o.n. ($\chi^2 = 7{\cdot}61$, $P < 0{\cdot}01$ at d.f. = 1) and in the p.v.n. ($\chi^2 = 5{\cdot}45$, $P < 0{\cdot}025$ at d.f. = 1). Most affected cells responded within 3 min and reached minimum (or maximum) frequency approximately 3–4 min after perfusion with a.n.p.-containing medium. The sign of the response did not depend on initial firing rate. After washing out the a.n.p. with control medium, firing rate recovered to pre-treatment values in 5–15 min.

To investigate the dose–response relationship, some neurones in the p.v.n. and a.v.3.v. were tested at different a.n.p. concentrations ranging from 10^{-12} to 10^{-6} M. Fig. 3 shows representative examples of the effects of a.n.p. at concentrations from 10^{-12} to 10^{-6} M. The responses of all seven cells (two p.v.n. and five a.v.3.v. cells) tested are summarized in Fig. 4 which shows the percentage change in firing rate from the resting value. As a.n.p. concentration increased, the firing rates of all seven cells generally decreased. A single a.v.3.v. neurone was excited at low concentrations of a.n.p. (less than 10^{-8} M) but inhibited at high concentrations of a.n.p. (10^{-7} and

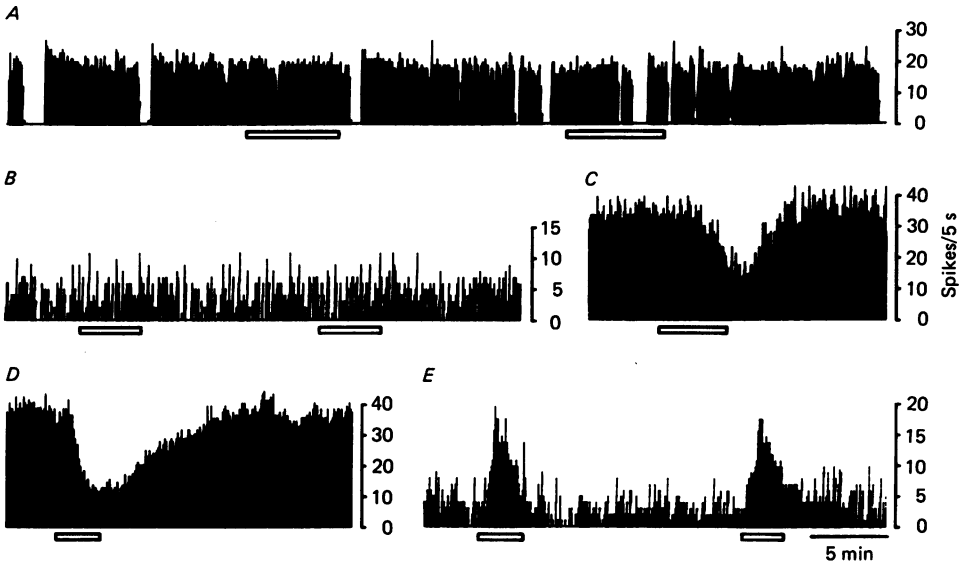

Fig. 2. The effects of a.n.p. in the s.o.n., p.v.n. and a.v.3.v. *A* and *B* show a phasic firing and a slow irregular firing s.o.n. cell respectively. Neither neurone was responsive to application of a.n.p. (10^{-7} M; indicated by open bars). *C* and *D* show inhibitory responses of neurones following application of a.n.p. (10^{-7} M; open bars) in the p.v.n. and a.v.3.v., respectively. *E* shows excitatory responses of a p.v.n. cell to application of a.n.p. (10^{-7} M; open bars).

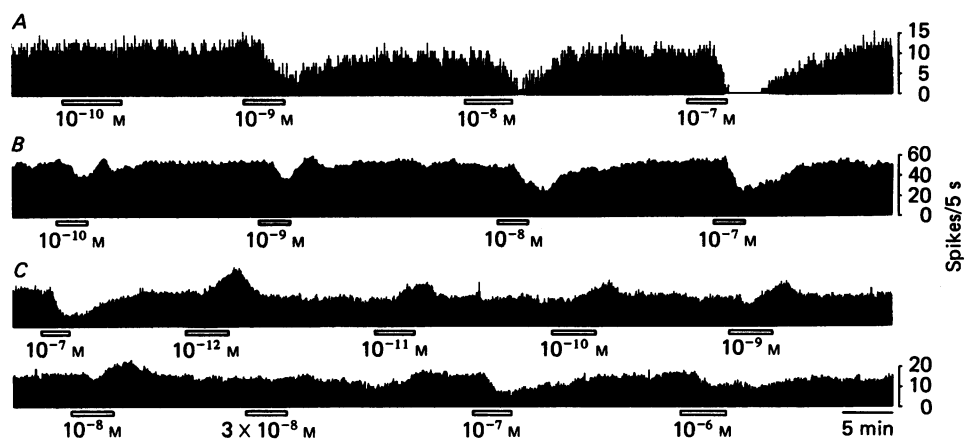

Fig. 3. Records to show graded responses of p.v.n. and a.v.3.v. neurones to different concentrations of a.n.p. *A* and *B* show rate-meter records of successive responses of neurones to increasing concentrations of a.n.p. (10^{-10} to 10^{-7} M) in the p.v.n. and a.v.3.v., respectively. At a concentration of 10^{-10} M-a.n.p., the p.v.n. cell scarcely showed an inhibitory response to a.n.p., while the a.v.3.v. cell showed a clear inhibitory response to a.n.p. *C* shows rate-meter records of the responses of another a.v.3.v. cell to different concentrations of a.n.p. (10^{-12} to 10^{-6} M). The neurone showed excitatory responses to a.n.p. at low concentrations (less than 10^{-8} M) but inhibitory responses to a.n.p. at high concentrations (10^{-7} and 10^{-6} M).

10^{-6} M; Figs. 3 and 4). The threshold a.n.p. concentrations required to evoke inhibitory responses of the p.v.n. and a.v.3.v. were approximately 10^{-10} and 10^{-11} M respectively. The inhibitory effects of a.n.p. were reversible and tachyphylaxis was seldom observed with repeated application of the peptide. The results indicated that most responsive cells showed inhibitory responses to application of a.n.p. in the p.v.n. and a.v.3.v.

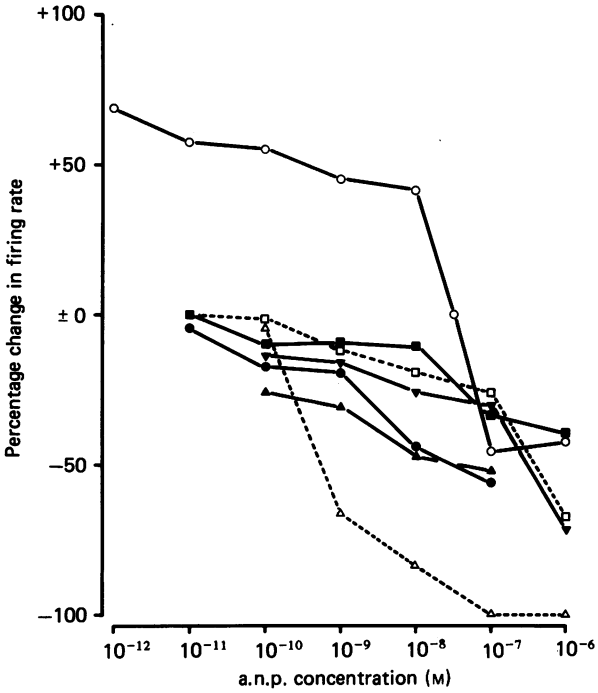

Fig. 4. Dose–response relationships of p.v.n. and a.v.3.v. neurones to a.n.p. The two broken lines show the dose–response curves of two p.v.n. neurones which were inhibited by a.n.p. and whose threshold concentration required to evoke the inhibitory responses was approximately 10^{-10} M. The five continuous lines show the dose–response curves of five a.v.3.v. neurones. The firing rate of each a.v.3.v. neurone generally decreased with increasing a.n.p. concentration. Only one a.v.3.v. neurone showed dose-dependent reversal responses to a.n.p., that is to say, excitatory responses at the low concentrations but inhibitory responses at the high concentrations. The threshold concentration to evoke inhibitory responses in the a.v.3.v. was approximately 10^{-11} M.

Effects of atrial natriuretic polypeptide on antidromically activated paraventricular nucleus neurones

We recorded fifteen neurones in the magnocellular portion of the p.v.n. which were antidromically activated by electrical stimulation of the area dorsolateral to the column of the fornix. The criteria for identifying an antidromically activated action potential were, as described by Dudek *et al.* (1980), constancy in latency at threshold and the ability to follow consecutive suprathreshold pulses at frequencies above 125 Hz. Current thresholds for antidromic activation ranged from 0·15 to 0·80 mA (0·43 ± 0·08 mA; mean ± S.E. of mean). Antidromic invasion occurred with a constant latency varying from 0·5 to 2·8 ms (1·4 ± 0·2 ms). Based on an average distance of

1·2 mm between stimulating and recording electrodes, the conduction velocity was calculated to be 0·8 m/s. Spontaneous discharge was present in ten (67%) of the fifteen cells. The mean firing rate of the spontaneously firing cells was 1·5±0·5 spikes/s. Two (20%) of the spontaneously firing cells showed inhibitory responses following application of a.n.p. (10^{-7} M) and the remaining cells were unaffected.

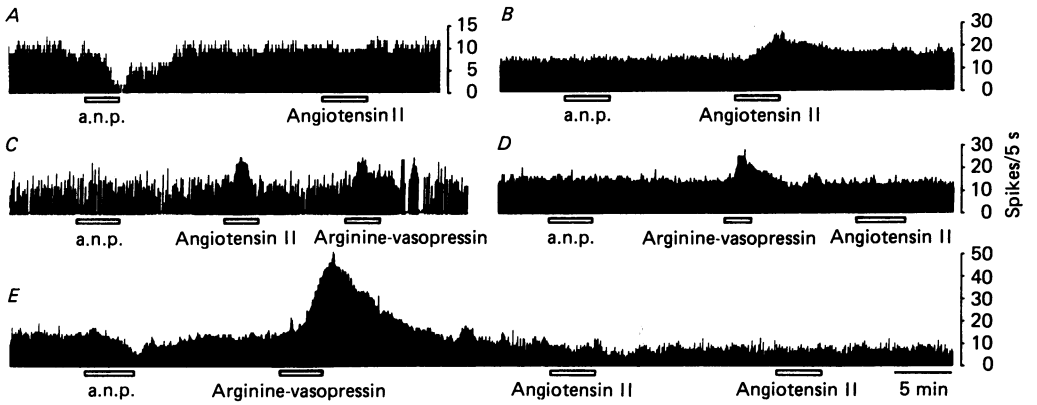

Fig. 5. Effects of peptides on p.v.n. and a.v.3.v. neurones. *A* and *B* show responses of single a.v.3.v. cells following application of a.n.p. (10^{-7} M) and angiotensin II (10^{-7} M). *C*, *D* and *E* show responses of single p.v.n. cells following application of a.n.p. (10^{-7} M), antiotensin II (10^{-7} M) and arginine-vasopressin (10^{-7} M). Note that no neurone showed both an inhibitory response to a.n.p. and an excitatory response to angiotensin II.

Effects of atrial natriuretic polypeptide and angiotensin II on anteroventral third ventricle neurones

We applied both a.n.p. and angiotensin II to single neurones in the a.v.3.v., where the existence of angiotensin II-sensitive cells had been reported (Gronan & York, 1978; Knowles & Phillips, 1980; Okuya *et al.* 1987). Of the thirty-six cells tested with both peptides at 10^{-7} M, eleven showed inhibitory responses to a.n.p., nine showed excitatory responses to angiotensin II and one was inhibited by both a.n.p. and angiotensin II. Almost all responsive neurones showed either inhibitory responses to a.n.p. or exitatory responses to angiotensin II, but never both. Neurones which showed inhibitory responses to a.n.p. were not excited by aniotensin II (Fig. 5*A*), while neurones which showed excitatory responses to angiotensin II were not inhibited by a.n.p. (Fig. 5*B*). Thus no single neurone showed both inhibitory responses to a.n.p. and excitatory responses to angiotensin II in the a.v.3.v.

Effects of atrial natriuretic polypeptide, angiotensin II and arginine-vasopressin on paraventricular nucleus neurones

Since arginine-vasopressin is known to have excitatory effects on neurones in the p.v.n. (Inenaga & Yamashita, 1986), we applied arginine-vasopressin in addition to a.n.p. and angiotensin II to single p.v.n. neurones. Of the forty cells tested with each peptide at 10^{-7} M, which included ten antidromically activated neurones, seven showed inhibitory responses to a.n.p., fourteen were excited by angiotensin II and twenty were excited by arginine-vasopressin. However, one was excited by a.n.p.,

two were inhibited by angiotensin II and three were inhibited by arginine-vasopressin. To summarize, a.n.p. showed mainly inhibitory effects on p.v.n. cells while both angiotensin II and arginine-vasopressin exerted excitatory effects. No neurones showed both inhibitory responses to a.n.p. and excitatory responses to angiotensin II (Fig. 5*C*), but four neurones showed both inhibitory responses to a.n.p. and excitatory responses to arginine-vasopressin (Fig. 5*E*).

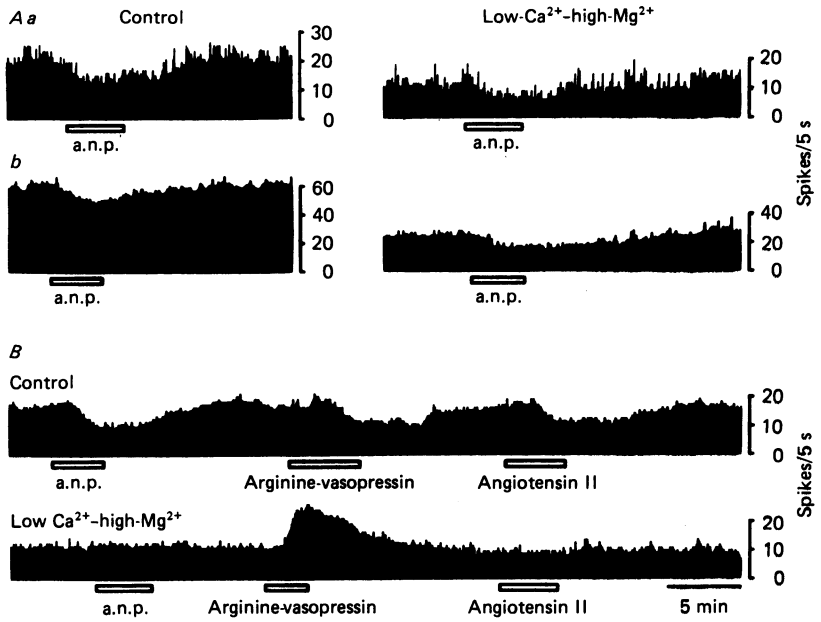

Fig. 6. Rate-meter records showing the effects of depression of synaptic transmission on the responses of p.v.n. and a.v.3.v. cells to application of peptides. *A a* and *b* show inhibitory responses of neurones to application of a.n.p. (10^{-7} M) in the p.v.n. and a.v.3.v., respectively. Inhibition was seen both on the left in a control perfusing medium and on the right in a low-Ca^{2+} and high-Mg^{2+} medium. *B* shows responses of an antidromically activated p.v.n. neurone to a.n.p. (10^{-7} M), arginine-vasopressin (10^{-7} M) and angiotensin II (10^{-7} M) in a control perfusing medium (the upper trace) and in a low-Ca^{2+} and high-Mg^{2+} medium (the lower trace). Note the disappearance of the responses to both a.n.p. and angiotensin II and the clear excitatory responses to arginine-vasopressin in a low-Ca^{2+} and high-Mg^{2+} medium.

Effects of peptides on paraventricular nucleus and anteroventral third ventricle neurones with depressed synaptic transmission

A low-Ca^{2+} and high-Mg^{2+} medium was used in an attempt to block synaptic transmission (Inenaga & Yamashita, 1986). About 15 min after the control perfusing medium had been changed to a low-Ca^{2+} and high-Mg^{2+} medium, a.n.p. (10^{-7} M) was applied to neurones which had earlier been inhibited by a.n.p. (10^{-7} M). Five (probably parvocellular) p.v.n. and five a.v.3.v. neurones which had shown inhibitory responses to a.n.p. in control perfusing medium were also inhibited after changing to the low-Ca^{2+} and high-Mg^{+2} medium (Fig. 6*A*). By contrast (Fig. 6*B*), two antidromically activated neurones, presumably magnocellular neurones, which had been inhibited by a.n.p. (10^{-7} M) and angiotensin II (10^{-7} M) in control perfusing medium

(Fig. 6*B*, control) were not responsive to a.n.p. or angiotensin II after changing the medium. The same cells which had been inhibited by arginine-vasopressin (10^{-7} M) in control medium (Fig. 6*B*, control) were, however, excited by arginine-vasopressin after changing the medium. Two other antidromically activated neurones, which had been excited by angiotensin II, also showed excitatory responses to angiotensin II in the low-Ca^{2+} and high-Mg^{2+} medium and two other antidromically activated neurones were excited by arginine-vasopressin in both media (not illustrated). The results indicate that some p.v.n. and a.v.3.v. cells are directly sensitive to a.n.p. and that some p.v.n. magnocellular neurones may also be directly sensitive to angiotensin II and/or arginine-vasopressin.

DISCUSSION

The concentration of a.n.p. used in the experiments ranged from 10^{-12} to 10^{-6} M and the threshold concentrations required to evoke inhibitory responses of the p.v.n. and a.v.3.v. neurones were approximately 10^{-10} and 10^{-11} M, respectively. The plasma a.n.p. concentration of rats is known to be 2×10^{-10} to 4×10^{-10} M (Tanaka *et al.* 1984). Therefore, it seems reasonable to suggest that the threshold concentrations found in this study lie in a range which is physiologically meaningful.

Most of responsive neurones in the p.v.n. and a.v.3.v. showed inhibitory responses to a.n.p. and responded to a.n.p. in a dose-dependent manner. However, we observed that three neurones were excited by application of a.n.p. at a concentration of 10^{-7} M, and that one neurone which was inhibited by application of a.n.p. at 10^{-7} M was excited at lower concentrations of a.n.p. (less than 10^{-8} M; see Fig. 3*C*). Another neurone, in Fig. 3*B*, which was inhibited by a.n.p. might have weak excitatory responses superimposed on the inhibitory responses at lower concentrations of a.n.p. (10^{-10} and 10^{-9} M). In the above five cases, it was impossible to determine whether the responses to a.n.p. were due to a direct or indirect effect, since these particular responses were encountered so rarely that experiments with depressed synaptic transmission could not be performed.

After the perfusion medium was changed to have a low concentration of Ca^{2+} and a high concentration of Mg^{2+}, the spontaneous activity of neurones in both the p.v.n. and a.v.3.v. was substantially reduced, implying a decreased synaptic drive to the cells, but they nevertheless continued to show some activity probably because a low Ca^{2+} concentration facilitates the spontaneous activity of the neurones (Pittman *et al.* 1981). However, the same low-Ca^{2+} and high-Mg^{2+} medium blocks synaptic transmission in the p.v.n. (Inenaga & Yamashita, 1986) so that most synaptic input is probably eliminated or strongly depressed in the modified medium. The slices were trimmed to reduce synaptic drive further but, despite this, in most cells the a.n.p.-induced responses in the low-Ca^{2+} and high-Mg^{2+} medium were similar to those in control medium. The two antidromically activated neurones inhibited by a.n.p. in control medium which did not respond to a.n.p. in the low-Ca^{2+} and high-Mg^{2+} medium were exceptional. It is thus probable that some p.v.n. and a.v.3.v. cells are themselves sensitive to a.n.p. and that a proportion of p.v.n. magnocellular neurones may receive some synaptic inputs from a.n.p.-sensitive cells located in or near the p.v.n.

Immunohistochemical studies have demonstrated that a.n.p.-containing neurones

form a widespread network throughout parts of the brain that are known to maintain the body fluid homeostasis, such as the preoptic–hypothalamic area (Saper *et al.* 1985; Jacobowitz *et al.* 1985; Kawata *et al.* 1985). Moreover, Kawata *et al.* (1985) have demonstrated that the largest accumulation of a.n.p.-immunoreactive perikarya and varicose fibres is contained in the a.v.3.v. and that there are also many a.n.p.-immunoreactive varicose fibres in the p.v.n., particularly in the periventricular and peripheral parts of the nucleus, while few fibres are found in the s.o.n. This is consistent with our results (1) that the proportion of cells inhibited by a.n.p. was significantly higher in the a.v.3.v. than in the s.o.n. and p.v.n.; (2) that none of fifteen s.o.n. neurones, most of which were magnocellular neurones, were inhibited by a.n.p.; and (3) that in the p.v.n. and a.v.3.v., the inhibitory effect of a.n.p. persisted on most but not the two antidromically activated p.v.n. neurones despite depressed synaptic transmission. Thus, a.n.p. may exert an inhibitory influence on neurones in the p.v.n. and a.v.3.v. acting directly on specific receptors as a neurotransmitter or neuromodulator but it probably does not have a direct effect on magnocellular neurones in either s.o.n. or p.v.n.

Although we have tested only seven neurones, the present study demonstrated in all tested cases that a.v.3.v. neurones were more sensitive to a.n.p. than p.v.n. neurones and that the threshold concentration for a.n.p. to evoke inhibitory responses in the a.v.3.v. was comparable to the plasma concentration of a.n.p. in rats. Blood-borne peptides are thought to enter and to affect the central nervous system through circumventricular organs which lack a blood–brain barrier. Thus it is possible that neurones in the a.v.3.v., especially in the organum vasculosum of the lamina terminalis, could be influenced by blood-borne a.n.p.

It has been reported that angiotensin II-immunoreactive perikarya and varicose fibres are found in the p.v.n. and a.v.3.v. (Lind, Swanson & Ganten, 1985). Therefore we compared the effect of a.n.p. with that of angiotensin II at the single neurone level in the p.v.n. and a.v.3.v. Since Inenaga & Yamashita (1986) demonstrated that arginine-vasopressin has excitatory effects on neurones in the p.v.n., we also applied arginine-vasopressin. As expected, a.n.p. exerted opposite effects to those of angiotensin II and arginine-vasopressin, that is, a.n.p. showed mainly inhibitory effects while angiotensin II and arginine-vasopressin exerted mainly excitatory effects. Perhaps surprisingly no neurones showed both inhibitory responses to a.n.p. and excitatory responses to angiotensin II in the p.v.n. and a.v.3.v., but there were some which showed both inhibitory responses to a.n.p. and excitatory responses to arginine-vasopressin in the p.v.n. Both a.n.p.- and angiotensin II-immunoreactive cells and fibres are similarly distributed in the p.v.n. and a.v.3.v., but the cells could be clearly divided into two groups, which are either a.n.p. sensitive or angiotensin II sensitive in both the p.v.n. and a.v.3.v., while arginine-vasopressin could affect either cell group in the p.v.n. Another new finding in the present study is that angiotensin II excited some neurones in the parvocellular portion of the p.v.n.

With depressed synaptic transmission, four antidromically activated neurones (two excited and two inhibited in the control perfusing medium) were all excited by application of arginine-vasopressin. Thus arginine-vasopressin probably has a direct excitatory effect on some magnocellular neurones in the rat p.v.n. Results obtained by intracellular recordings *in vitro* (Abe, Inoue, Matsuo & Ogata, 1983) suggest that

magnocellular neurones in the s.o.n. of guinea-pigs are mainly inhibited by application of lysine-vasopressin. It is not clear why the results differ but there are a number of possible explanations. First, vasopressin may have different effects in different species, i.e. guinea-pigs and rats; secondly the characteristics of the magnocellular neurones may be different in the s.o.n. and p.v.n.; and thirdly, the neurones which were antidromically activated in the magnocellular portion of the p.v.n. may not have been oxytocin or vasopressin neurones but neurones containing other peptides such as corticotropin releasing factor (Lengvári, Liposits, Vigh, Schally & Flerkó, 1985). To determine which of these suggestions is correct it is probably necessary to test the effects of arginine-vasopressin on more antidromically activated neurones in the p.v.n., since we have tested only ten spontaneously firing cells which were antidromically activated.

There is clear morphological evidence that efferents from the a.v.3.v. terminate on s.o.n. and p.v.n. neurones (Swanson & Sawchenko, 1983) and electrophysiological evidence that cells in the p.v.n. project directly to preganglionic cell groups of both the parasympathetic (Kannan & Yamashita, 1985) and sympathetic (Yamashita, Inenaga & Koizumi, 1984) divisions of the autonomic nervous system. It is thus possible that elements in the a.v.3.v. detect homeostatically important information originating from circulating blood, some of which may be transmitted by neurones containing a.n.p., and send the information to the s.o.n. and p.v.n. to control arginine-vasopressin release and the activity of the autonomic nervous system which in turn contribute to the maintenance of body fluid homeostasis.

While the details of such proposed mechanisms are unclear, we may conclude that a.n.p. has central actions which include a direct inhibitory effect on some neurones in the p.v.n. and a.v.3.v. but not on magnocellular neurones in either the s.o.n. or p.v.n., and that a.n.p. has an opposite action to angiotensin II and arginine-vasopressin at the single neurone level in both the p.v.n. and a.v.3.v.

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