Neurophysiological Responses to Angiotensin-(1–7)

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The aim of this study was to investigate the action of the heptapeptide angiotensin-(1–7) on the spontaneous activity of paraventricular neurons using microiontophoresis. Recent immuno-cytochemical investigations have shown that this product of angiotensin I is predominantly located in cells and fibers of the forebrain and brain stem. Our results show that most neurons in the paraventricular nucleus are excited by angiotensin-(1–7) at a dose of 50–80 nA. In comparison with angiotensin II or angiotensin III, the onset of response and the occurrence of the maximal effect were significantly delayed. With higher doses of angiotensin-(1–7), there was a decrease in latency and a dose-dependent increase in firing frequency. Of all the angiotensin compounds tested, angiotensin III was the most potent. Preliminary results obtained with an angiotensin antagonist show that the action of angiotensin II, angiotensin III, and angiotensin-(1–7) is blocked by the angiotensin receptor subtype 2 antagonist CGP 42112A. Because the angiotensin-(1–7) system in the brain is associated with central vasopressinergic pathways, vasopressin was tested in a similar way. Neurons in the paraventricular nucleus that were excited by iontophoretically applied angiotensins showed a weak response to vasopressin. Occasionally, a small excitatory action was observed. Our results support the hypothesis that the heptapeptide angiotensin-(1–7) is a biologically active neuropeptide. The data also suggest that amino terminal fragments of angiotensin II are not inactive degradation products.

Angiotensin (Ang) peptides exert much of the regulatory actions on the cardiovascular system and hydromineral balance via specific brain sites. This neuronal system consists of interconnected structures located primarily in the perithird ventricular area, the hypothalamus, and the brain stem. The octapeptide Ang II traditionally has been considered the active form of the renin-angiotensin system in that it regulates thirst, sodium appetite, sympathetic tone, and vasopressin release. In recent years, however, several studies have shown that fragments containing the C-terminal sequence of Ang II mimic actions of the parent hormone.

In the course of establishing the product of hydrolysis of 125I-Ang I by brain tissue homogenates, Schiavone and coworkers found that a major component of the latter was the N-terminal heptapeptide Ang-(1–7). Investigations into the biological activity of this Ang fragment revealed a potent stimulatory effect on vasopressin secretion from rat hypothalamo-neurohypophysial explants and on prostaglandin synthesis in the vasa deferentia of rabbits. In contrast, Ang-(1–7) possesses no dipsogenic reaction or direct constrictor activity. A recent publication describes the neuroanatomical distribution of Ang-(1–7) using a specific polyclonal antibody. Ang-(1–7) immunoreactivity was found in restricted areas of the forebrain and the brain stem. Likewise, Campagnole-Santos et al. observed that Ang-(1–7) expressed biological activity through receptor-mediated actions in the dorsal medulla oblongata.

The distribution of Ang-(1–7) in the rat brain, together with the demonstration of vasopressin secretion in response to this peptide, led to the hypothesis that this fragment functions as a neuromodulator. To test this possibility, we examined the effect of the heptapeptide Ang-(1–7) on the activity of paraventricular neurons using microiontophoresis.

Methods
Experiments were performed on 13 female adult Wistar-Kyoto rats (250–300 g) anesthetized with an...
intraperitoneal injection of 50 mg/kg thiopentane sodium (Pentothal, Abbott laboratories, Zug, Switzerland). The tracheotomized animals were mounted in a stereotaxic frame, and their body temperature was maintained at 38°C by a regulated heating pad. After a part of the skull was removed, microelectrodes were inserted into the brain and placed stereotaxically in the paraventricular nucleus. At the end of the testing period, a fast green dye (FCF) marker was injected at the recording site to locate the electrode site histologically.

Extracellular spontaneous activity was recorded using conventional recording equipment through one barrel of a five-barrel glass micropipette containing 2 M NaCl with a tip diameter of approximately 2 μm. The other barrels of the micropipette contained the compounds to be ejected microiontophoretically with appropriate cationic or anionic currents: 1) [Ile]Ang II (Sigma Chemical Co., St. Louis) prepared as a 10^-3 M solution in distilled water, final pH 4.5; 2) [Ile]Ang III (Sigma), 10^-3 M, pH 4.5; 3) [Des-Phe]Ang II, Ang-(1-7), synthesized by Dr. M. Khosla, Cleveland, Ohio, 10^-3 M, pH 4.5; 4) [Arg]vasopressin (Sigma), 10^-3 M, pH 4.5; 5) L-glutamate (BDH Ltd., Poole, UK), 1 M, pH 7.5; 6) acetylcholine chloride (Fluka Chemie AG, Buchs, Switzerland), 0.5 M, pH 3.5-4.0; and 7) the Ang II antagonist CGP 42112A, kindly provided by Dr. Marc deGasparo (CIBA-GEIGY Basel Ltd., Basel, Switzerland), 10^-4 M, pH 4.5.

Compensation current through a channel containing sodium chloride was always used to prevent any direct current effects.

Results

Results were obtained from 35 paraventricular neurons. The majority of the neurons studied fired spontaneously at a relatively low rate, ranging from 0.2 to 2 spikes/sec. A small number of silent neurons were excited only after administration of either glutamate (10–50 nA) or acetylcholine (50–100 nA). Iontophoretic studies using angiotensins in the region of the paraventricular nucleus showed that most of the cells were excited by both Ang II and Ang III. An important technical consideration for microiontophoresis is the determination of the actual rate of microiontophoretic release for a given amount of ejection current (expressed in nanoamperes). Although considerable variation was observed between microelectrodes, the mean rate of Ang II and Ang III release was very similar: Ang II, 41.5±4.8 fmol/nA/min; Ang III, 45.1±8.0 fmol/nA/min. In all cells in which comparative recordings were made, Ang III usually was more potent than Ang II (Figure 1). Ang III responses were characterized by a lower threshold (Ang III average, 15 nA; Ang II, 28 nA) and a larger maximal response per nanoampere of current applied.

In a second series of experiments, we tested the effects of Ang-(1-7) in comparison to the excitatory effects of Ang III described earlier. Out of a total of 32 cells that responded to iontophoretically applied Ang III, 28 neurons also responded to Ang-(1-7). The effect was always excitatory, although the onset of responses and the occurrence of maximal responses were significantly delayed (Figure 1). In a comparative study using equal amounts (50 nA) of Ang III and Ang-(1-7), the average latency of Ang III was 20 seconds and that of Ang-(1-7) was 108 seconds. Correspondingly, the maximal responses were obtained after 48 seconds for Ang III and 142 seconds for Ang-(1-7). With higher doses of Ang-(1-7), we observed a decrease in latency and a dose-dependent increase in firing frequency. Figure 2 shows the statistical analysis of the maximal responses of Ang III and Ang-(1-7) ejected at doses of 40–50 nA each.

Because it has been shown that the Ang-(1-7) system in the brain is associated with central vasopressinergic pathways, we also tested the action of vasopressin on paraventricular neurons. Neurons located in the magnocellular part of the paraventricular nucleus usually responded to the ejection of angiotensins. The same neurons did not react to vasopressin, however (Figure 1). A small increase in activity was observed only occasionally. In contrast, a
clear excitatory action of vasopressin was detected on neurons located in the lateral area adjacent to the magnocellular part of the paraventricular nucleus. In further experiments, the peptidergic Ang II antagonist CGP 42112A,24 which has been shown to block Ang receptor subtype II (or type A) was tested iontophoretically on the excitatory action of Ang II, Ang III, and Ang-(1-7). On all 12 neurons tested, the excitatory action of all three peptides was blocked in a reversible manner. The onset of antagonistic action was seen within 1–2 minutes. Cells again were able to be excited by the angiotensins within a similar period after cessation of ejection. We also observed a decrease in spontaneous unit firing on most of the cells (eight out of 12 neurons), suggesting that part of the spontaneous activity is maintained by endogenous brain Ang (Figure 3). The antagonistic action seems to be specific on angiotensins, because neither the excitatory responses to glutamate nor to acetylcholine were affected. Furthermore, the use of the cholinergic antagonist atropine affected the acetylcholine-stimulated responses only.

Discussion

The experiments described above were done to test the sensitivity of paraventricular neurons to the N-terminal heptapeptide Ang-(1–7). Data obtained from immunocytochemical investigations using a specific polyclonal antibody have shown that this heptapeptide is located in cells and fibers associated with central vasopressinergic pathways.19 This, together with the fact that Ang-(1–7) stimulates the secretion of vasopressin from hypothalamic brain explants15 and increases the synthesis of prostaglandin in the vas deferentia,16 led to the hypothesis that the predominant metabolite of Ang I, Ang-(1–7), is biologically active and may play a role as a neuromodulator. The clear activation of paraventricular neurons by microiontophoretic administration of Ang-(1–7) indicates that this substance interacts with a possible Ang receptor in this area. This would seem all the more likely because it has been shown that a subtype 2 receptor antagonist blocks the excitatory action of Ang II, Ang III, and Ang-(1–7) in a reversible manner.

Differences in latency may account for variations in the diffusion rate between the peptides or separate sites of action (i.e., presynaptic versus postsynaptic mechanisms). Alternatively, the excitatory effects of Ang-(1–7) may be mediated by the release of prostaglandin through activation of non-Ca2+-dependent cellular phospholipase. This possibility agrees with the recent finding that the potent stimulatory action of Ang-(1–7) on prostaglandin synthesis in astrocytes is not associated with mobilization of Ca2+ from intracellular stores.25 Direct demonstration of an excitatory action of Ang-(1–7) on magnocellular neurons of the paraventricular nucleus are novel findings, providing evidence at a cellular level for a modulatory action of this heptapeptide on the regulation of vasopressin secretion. The observation that
the excitatory actions of Ang-(1–7) and other angiotensins occurred in subpopulations of the neurons that do not respond to the similar application of vasopressin further characterized the neuronal substrate involved in these actions.

A further obvious explanation for the delayed response could be that the peptide is being metabolized, and perhaps the metabolite is the active moiety.

There is the likelihood that aspartic acid from the N-terminus is being cleaved and may be giving the response. Therefore, it will be necessary to test Ang-(2–7) to answer this question.

In summary, these basic studies show a modulatory action of Ang-(1–7) on the spontaneous activity of vasopressinergic neurons in the rat.

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