Pharmacology Biochemistry and Behavior, Vol. 1, pp. 223–226. ANKHO International Inc., 1973. Printed in the U.S.A.

BRIEF COMMUNICATION

Effects of Angiotensin Applied Electrophoretically on Lateral Hypothalamic Neurons[1]

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(Received 15 January 1973)

WAYNER, M. J., T. ONO AND D. NOLLEY. *Effects of angiotensin applied electrophoretically on lateral hypothalamic neurons.* PHARMAC. BIOCHEM. BEHAV. 1(2) 223–226, 1973.–The effects of angiotensin II applied by means of electrophoresis through multibarreled glass capillary electrodes on the extracellularly recorded discharge frequencies of brain cells were determined. A total of 148 neurons in 21 female hooded rats anesthetized with urethan and chloralose were tested. Results indicate two types of neurons within the lateral hypothalamus (LH) which are affected differently by angiotensin II; those of the medial forebrain bundle (LH-MFB) which increase in discharge frequency, and another type at the ventral border of the LH which decrease in discharge frequency. Neurons of the zona incerta were similar to those of the medial forebrain bundle. All of the Na sensitive cells of the LH-MFB and the zona incerta were also sensitive to angiotensin II. Neurons of the LH, LH-MFB and zona incerta have a relatively low threshold to angiotensin II. At higher doses the effect appears to be more nonspecific and one third of the thalamic cells tested also responded. Only one of the 13 neurons in the cerebral cortex was affected. Angiotensin II in small quantities might therefore influence drinking because of a specific effect on Na sensitive neurons of the LH.

Angiotensin II Microelectrophoresis Lateral hypothalamus Drinking Zona incerta Hypothalamus

ANGIOTENSIN is a substance of considerable current interest because it appears to be involved in normal body fluid regulation by stimulating the release of aldosterone and antidiuretic hormone [4] and by a direct action on hypothalamic neurons implicated in the control of drinking [3]. Results which indicate that the preoptic region, anterior hypothalamus, lateral hypothalamus, and septal region are differentially sensitive to angiotensin might be confounded by the spread of the administered fluid along the shaft of the indwelling chronic cannulae into the ventricles with possible action at some other tissue sites [5,10]. Since angiotensin applied intraventricularly elicits drinking [2] and there is no evidence that it crosses the blood brain barrier [9], an indirect route to central neurons via the choroid plexuses, cerebrospinal fluid and ventricles, particularly into the walls of the third ventricle [12] seems very likely. In addition, both angiotensin and carbachol when injected in the midline region of the rat brain produce drinking [11]. These results raise some serious doubts concerning the functional significance of the renin–angiotensin system in the elicitation of drinking and raise the important question of specific sensitivity of central neurons to angiotensin. Two brief reports have appeared recently in which the sensitivity of hypothalamic neurons to angiotensin was determined by more direct means [6,8]. The purpose of the present experiment was to examine in greater detail the effects of valine^5-angiotensin II on the discharge frequency of lateral hypothalamic neurons of the rat when applied microelectrophoretically. Results indicate two types of neurons within the lateral hypothalamus (LH) which are affected differently by angiotensin.

METHOD

Animals

Experiments were performed on 21 female hooded rats, 250–290 g in weight, selected from our colony.

Procedures

Animals were anesthetized with a mixture of chloralose (50 mg/kg) and urethan (0.5 g/kg) administered intraperitoneally. Subsequent maintenance doses of chloralose

[1] This research was supported by NSF Grant GB-18414X and NIMH Grant 15473 and Training Grant MH-06969. We are also grateful to Dr. Albert J. Plummer of CIBA-GEIGY Corporation, Summit, New Jersey, who supplied us with the valine^5-angiotensin II.

[2] Dr. T. Ono expresses his gratitude to the Naito Research Foundation which paid his travel expenses to the U.S.A.

(10–20 mg/kg) and urethan (0.1–0.2 g/kg) were injected when necessary. In general the remainder of the procedures were as described previously [13]. Briefly, three barreled glass capillary microelectrode arrays were used. Extracellular single unit discharges were recorded through one barrel filled with 4 M NaCl, resistance varied from 5 to 25 MΩ. Action potentials were amplified, monitored visually, stored on magnetic tape, and analyzed by conventional means. The other two barrels were filled with angiotensin II (CIBA, 83% valine^5-angiotensin II and 17% ammonium acetate), 12–60 mg/ml in distilled water with a final pH of 5.5; and monosodium-l-glutamate, 2 M, pH of 8.0 (NaOH). Both substances were ejected in the vicinity of the tip mainly by outward currents supplied by a constant current source [14]. The resistances of these two barrels varied from 5 to 80 MΩ. The overall tip diameter of the three barreled array varied from 3–6μ. Possible direct electrical effects of the microelectrophoretic currents were evaluated on the basis of previously published criteria [1] and unreliable data were discarded. Tests performed with ammonium acetate alone in distilled water were negative and indicate that the effects reported here can be attributed to the angiotensin. The final electrode tip position within the brain for each experiment was determined by perfusing the animal with the electrode shaft in place. After fixation the electrode was removed and the brain was sectioned at 40 μ, stained with cresyl violet, and examined by means of a dissecting microscope.

TABLE 1

A SUMMARY OF THE EFFECTS OF ANGIOTENSIN II AND Na ON THE NEURONS OF FIVE DIFFERENT BRAIN SITES

Site	Angiotensin N	Angiotensin E	Angiotensin I	Angiotensin O	Na N	Na E	Na I	Na O
LH	17	0	9	8	2/9	0	1	1
LH-MFB	29	19	0	10	14/19	3	0	11
					10/10	0	0	10
Zona Incerta	23	16	1	6	16/16	4	0	12
					1/1	0	1	0
					3/6	0	0	3
Thalamus	66	18	7	41	18/18	0	0	18
					7/7	0	0	7
Cortex	13	0	1	12	2/12	0	0	2

RESULTS

The data on 148 cells from 5 different brain sites are summarized in Table 1. The number of cells, N, studied in each site is also included. Two different types of neuron were found in the LH; those definitely within the medial forebrain bundle (LH-MFB), and a different variety located about 0.5 mm more ventral and slightly more medial (LH) from the center of the lateral hypothalamus. Of 17 LH cells studied, nine were inhibited (I), none were excited (E), and eight were not affected (O) by the ejected angiotensin. Of the nine which were inhibited, two were tested with Na and in one the discharge frequency decreased (I) and the other was not affected. These cells of the lower LH have a relatively low spontaneous discharge frequency which make them particularly difficult to locate. Searching was facilitated by the electrophoretic application of glutamate through one of the capillaries. The effects of glutamate and angiotensin on one of these neurons are illustrated in Part B of Fig. 1. This cell had a spontaneous discharge frequency of about one every three seconds which was definitely increased by the ejection of glutamate which continued from the vertical arrow until the end of the fourth tracing. The inhibitory effects of angiotensin ejected by currents of 2, 5 and 20 nA are also illustrated. The duration of the angiotensin application is indicated by the horizontal line. Since three barreled electrodes were used and one capillary was filled with glutamate, it was impossible to test any one cell with both angiotensin and Na. The two cells tested for Na therefore did not have enhanced discharge frequencies due to glutamate.

Twenty-nine LH-MFB neurons were tested. Nineteen were excited by angiotensin, increased in discharge frequency, none were inhibited, and 10 were not affected. The effects of 10, 20, and 100 nA current applied through the angiotensin filled capillary are illustrated in Part A of Fig. 1. A dose related increase is obvious. A dose related after effect is also apparent. As these cells had a relatively high spontaneous discharge rate as compared to the lower LH, mean of 7.3 spikes per sec with a standard error of 1.4 spikes per sec, glutamate facilitation was not necessary and 14 out of the 19 excited cells were tested with Na. Three of these cells were excited by Na and 11 were not affected. Of the 10 not affected by angiotensin, all were not affected by Na.

In the zona incerta 23 neurons were tested. Of these, 16 were excited, one decreased in discharge frequency, and six were not affected. The mean spontaneous discharge rate was 6.6 spikes per sec with a standard error of 2.1 spikes per sec. Of the 16 which were excited by angiotensin, 4 increased in discharge frequency due to Na, and 12 were not affected. The one which was inhibited by angiotensin was also inhibited by Na. Three of the six cells not affected by angiotensin were also tested by Na and were not affected. The neurons of the LH, LH-MFB, and zona incerta have a low threshold to angiotensin with a required ejection current of less than 20 nA. Both an increase and decrease in discharge frequency are illustrated in Fig. 1. The Na sensitive effects had a higher threshold in general and required ejection currents of approximately 100 nA.

A relatively large number of thalamic cells were tested because all of the neurons studied were located along essentially the same electrode tract which resulted from the attempt to place each electrode tip in the same predetermined site. Of the 66 cells tested, 18 increased, 7 decreased in discharge frequency, and 41 were not affected. All of these neurons displayed high thresholds and required more than 50 nA of ejection current to produce an observable increase in frequency. All of the 18 which were increased

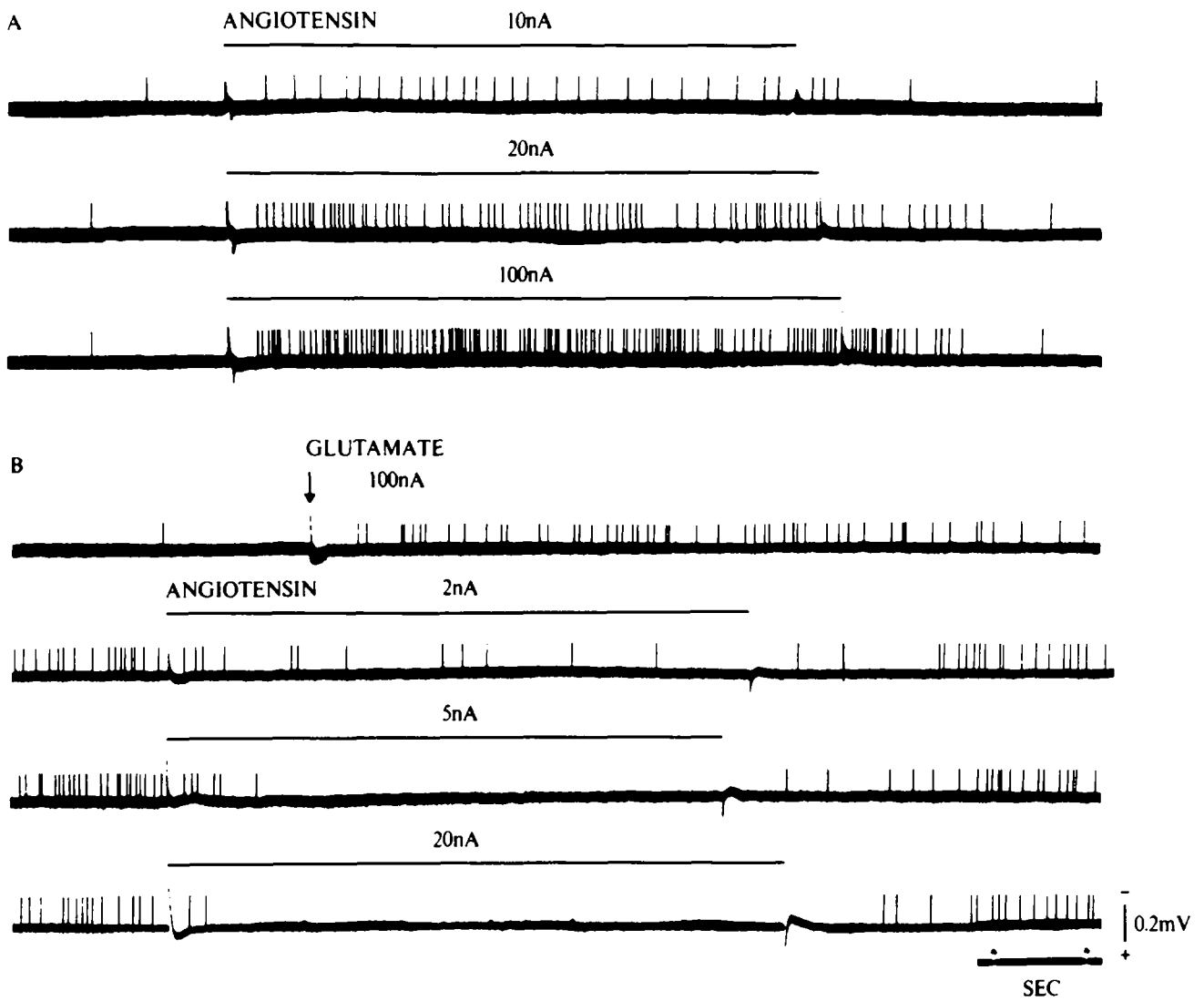

FIG. 1. A. A neuron of the LH-MFB region which increased in discharge rate during the ejection of 10, 20, and 100 nA of current. B. A neuron of the lower LH. The spontaneous discharge rate increased by the application of glutamate, from the downward pointing arrow until the end of the fourth tracing, and decreased by the ejections of 2, 5, 20 nA of angiotensin II as indicated by the horizontal solid lines.

by angiotensin were tested with Na and were not affected. The seven which decreased were also tested with Na and were not affected. In the cerebral cortex 13 cells were tested by angiotensin, one decreased in discharge frequency and 12 were not affected. The decrease in discharge frequency by the one cell required an ejection current of over 200 nA and it is unlikely that the effect can be attributed only to the angiotensin. Two cells were tested with Na and were not affected.

DISCUSSION

These somewhat tenuous results indicate that many cells of the LH and zona incerta are relatively more sensitive to angiotensin and Na than neurons of the thalamus and cerebral cortex. Many more cells are sensitive to angiotensin than Na and all Na sensitive cells are also sensitive to angiotensin. The low spontaneously active ventral LH neurons decrease in discharge frequency when angiotensin is applied; whereas, the cells of the LH-MFB increase in discharge rate. The neurons of the zona incerta are very similar in this respect to the LH-MFB region and possibly represent cells of the same population. The relatively crude histological methods which were utilized do not permit a clear differentiation between the termination of the LH and beginning of the zona incerta. Therefore angiotensin seems to have a specific effect on two types of neurons in the ventral LH and LH-MFB-zona incerta regions. With larger doses the effect is more nonspecific and many cells of the thalamus are also affected. All Na sensitive neurons of the LH-MFB-zona incerta were also sensitive to angiotensin. Therefore angiotensin in small quantities might influence drinking because of a specific effect on the LH Na sensitive neurons [7]. Although these neurons appear to be in the same general region where hypertonic NaCl and acetylcholine elicit drinking, the effects of angiotensin appear to be independent of the effects of acetylcholine [8]. Angiotensin also seems to have a predominantly excitatory action on neurons of the supraoptic nucleus which suggests a direct effect in the release of antidiuretic hormone [6].

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